A STUDY ON MICROBIOLOGICAL PROFILE OF SYMPTOMATIC CATHETER ASSOCIATED URINARY TRACT INFECTION IN AN INTENSIVE CARE UNIT SETUP IN A TERTIARY CARE HOSPITAL

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THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

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APRIL 2016

CERTIFICATE

This is to certify that this dissertation titled **"**A STUDY ON MICROBIOLOGICAL PROFILE OF SYMPTOMATIC CATHETER ASSOCIATED URINARY TRACT INFECTION IN AN INTENSIVE CARE UNIT SETUP IN A TERTIARY CARE HOSPITAL" is a bonafide record work done by DR. SWATI SAHAI during the period of her Post Graduate study from AUGUST 2013 to APRIL 2016 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 600003, in partial fulfillment of the requirement of **M.D MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R Medical University to be held in April 2016.

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DECLARATION

I declare that the dissertation entitled **"**A STUDY ON MICROBIOLOGICAL PROFILE OF SYMPTOMATIC CATHETER ASSOCIATED URINARY TRACT INFECTION IN AN INTENSIVE CARE UNIT SETUP IN A TERTIARY CARE HOSPITAL"*submitted by me for the degree of M.D. is the record work carried out by me during the period of October**2014 –** September**2015** under the guidance of* **Dr.S.Vasanthi, M.D.,** Professor of Microbiology, *Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2016.*

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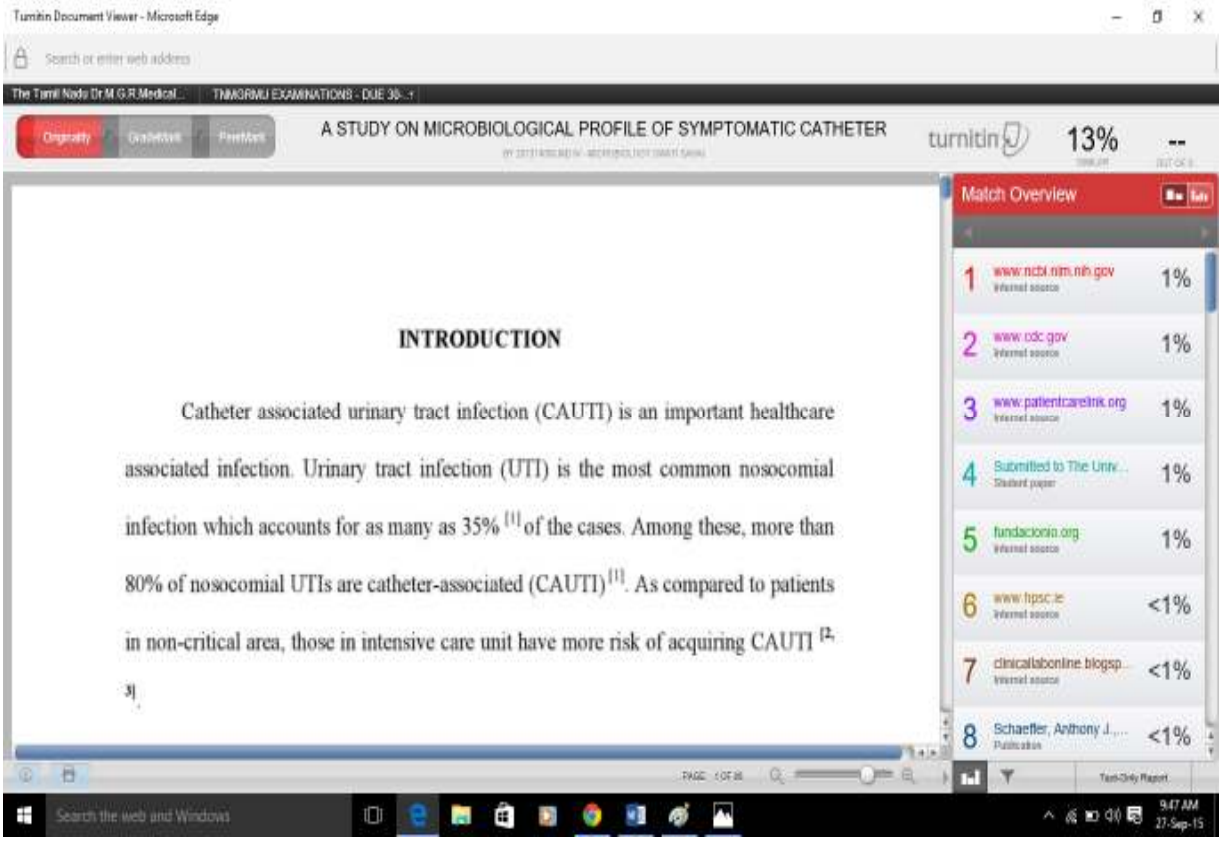
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INTRODUCTION
Catheter associated urinary tract infection (CAUTI) is an important healthcare associated infection. Urinary tract infection (UTI) is the most common nosocomial infection which accounts for as many as 35% [1] of the cases. Among these, more than 80% of nosocomial UTIs are catheter-associated (CAUTI) [1]. As compared to patients in non-critical area, those in intensive care unit have more risk of acquiring CAUTI [2, 3].

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A STUDY ON MICROBIOLOGICAL PROFILE OF SYMPTOMATIC CATHETER ASSOCIATED URINARY TRACT INFECTION IN AN INTENSIVE CARE UNIT SETUP IN A TERTIARY CARE HOSPITAL

ABSTRACT

Introduction: Catheter associated urinary tract infection (CAUTI) is an important healthcare associated infection. More than 80% of nosocomial UTIs are catheter-associated. Patients in intensive care unit have more risk of acquiring CAUTI.

Aim & Objectives: To determine the incidence rate of symptomatic catheter associated urinary tract infection in intensive care unit set-up, to isolate the organisms involved in symptomatic CAUTI, to determine the antimicrobial sensitivity pattern of the isolates, to determine the association of symptomatic CAUTI in relation to high risk factors

Materials & Methods: A total of 100 patients admitted in medical ICU and put on Foley's catheter were included in the study and followed up for the development of symptomatic CAUTI. The urine samples from the catheter were collected on day 1 and then on day 3,5,7,10,14 and every weekly till the patient was discharged, expired, catheter removed or developed bacteriuria. The samples were processed as per standard guidelines.

Results: Total 26 patients developed Symptomatic CAUTI, thereby the incidence being 26 %. *Pseudomonas aeruginosa* was the commonest isolate (25%) followed by *Klebsiella pneumoniae* (17.5%), *Escherichia coli, Enterococcus faecalis* and *Candida spp*. (15%) each. *P.stutzeri* comprised 5% and *Klebsiella oxytoca*, *P.fluorescens* and *Staphylococcus aureus* 2.5% each. High degree of antibiotic resistance was observed

.On the whole the bacterial isolates were more sensitive to piperacillin-tazobactam, imipenem and meropenem.

Conclusion: Development of CAUTI is common in critically ill patients Emphasis should be placed on good catheter management and reducing the duration of catheterization to reduce its incidence. Knowledge of resistant pattern can help in implementing proper antibiotic therapy and infection control policy.

Key words: catheter associated UTI, nosocomial

INTRODUCTION

Catheter associated urinary tract infection (CAUTI) is an important healthcare associated infection. Urinary tract infection (UTI) is the most common nosocomial infection which accounts for as many as 35% [1] of the cases. Among these, more than 80% of nosocomial UTIs are catheter-associated (CAUTI) [1]. As compared to patients in non-critical area, those in intensive care unit have more risk of acquiring CAUTI [2, 3].

Healthcare associated infection (HAI) has emerged as an important health problem throughout the world, causing significant mortality and morbidity [4]. HAI is defined as clinical infection that develops after 48 hours of admission to a hospital for treatment of a different initial illness. These infections were neither overtly present nor within the incubation period at the time of admission, and are often due to organisms endemic in the hospital [5].

If a patient has an indwelling catheter for 48 hours or more and then develops signs and symptoms of UTI, it is considered as symptomatic CAUTI. If the catheter was removed after 2 days and the UTI criteria is fulfilled on the day of removal or the next day, then also it is considered CAUTI. It is of 2 types: Symptomatic & Asymptomatic bacteremic CAUTI. Symptomatic CAUTI is considered when symptoms / signs consistent with UTI exists along with bacteriuria in a catheterized patient. [6] The signs and symptoms either are localized to the urinary tract or can include otherwise unexplained systemic manifestations, such as fever. The accepted threshold for bacteriuria varies from 10^3 cfu/mL to 10^5 cfu/mL [61].

The source of infection can either be endogenous or exogenous. The presence of meatal, rectal, or vaginal colonization by microorganisms causes endogenous infection. Exogenous infections occur during insertion or manipulation of the catheter and collecting system by health personnel [6]. Even after using closed, sterile drainage system and inserting the catheter aseptically, patients still acquire the infection [7].

A number of risk factors are implicated in the causation of CAUTI. These include female sex, old age, increased duration of hospital stay and catheterisation, impaired immunity, severe underlying illness, diabetes mellitus, renal dysfunction, incontinence, neurological/orthopaedic causes, disconnection of drainage system and faulty catheter care [6, 8].

The causative organisms include Gram negative bacilli (GNB), Gram positive bacteria and even Candida spp. Earlier the most common organism was *Escherichia coli*. Recent studies indicate a shift towards non-fermentative GNBs, *Enterococcus spp.* and *Candida spp*.

CAUTI can produce a wide range of complications. These include fever, urethritis and cystitis in mild cases and acute pyelonephritis, renal scarring, calculi formation and bacteremia in severe cases. It can also cause prostatitis, epididymitis, and orchitis in males. Other less common complications include septic arthritis, endocarditis, vertebral osteomyelitis, septic arthritis, etc.[6].

Due to intense microbial exposure, antimicrobial resistant organisms are frequently isolated in these cases. CAUTIs constitute a huge reservoir of antimicrobial resistance and inadvertent use of antibiotics increases the risk of cross-infections among catheterized patients. This results in prolonged hospital stay and increased cost of

healthcare, not only to the patients but also to the hospital. Ultimately there is overall increased morbidity and mortality.

Due to increase in prevalence of multidrug resistant isolates, early detection is crucial. This study will help in planning effective infection control policies and proper antimicrobial policy, thereby decreasing the incidence of antimicrobial resistance.

AIMS AND OBJECTIVES

1. To determine the incidence rate of symptomatic catheter associated urinary tract infection in intensive care unit set-up
2. To isolate the organisms involved in symptomatic CAUTI
3. To determine the antimicrobial sensitivity pattern of the isolates.
4. To determine the association of symptomatic CAUTI in relation to high risk factors

REVIEW OF LITERATURE

ANATOMY OF URINARY TRACT [9,10,11]

The urinary tract includes **kidneys, ureters, urinary bladder** and **urethra**. The role of the kidneys is excretion of the waste products of metabolism, maintenance of water and electrolyte balance of the body and the acid–base balance of the blood.

The kidneys are reddish brown, bilaterally paired organs present in retroperitoneum. The upper pole of each kidney lies opposite 12th thoracic vertebra and lower pole opposite 3rd lumbar vertebra. The right kidney lies slightly lower than the left kidney due to the presence of right lobe of the liver.

The **ureters** extend from the kidneys to the posterior surface of the urinary bladder. Each ureter measures about 25 cm long. Due to peristaltic contractions of the muscle coat, urine gets propelled along the ureter, assisted by the filtration pressure of the glomeruli.

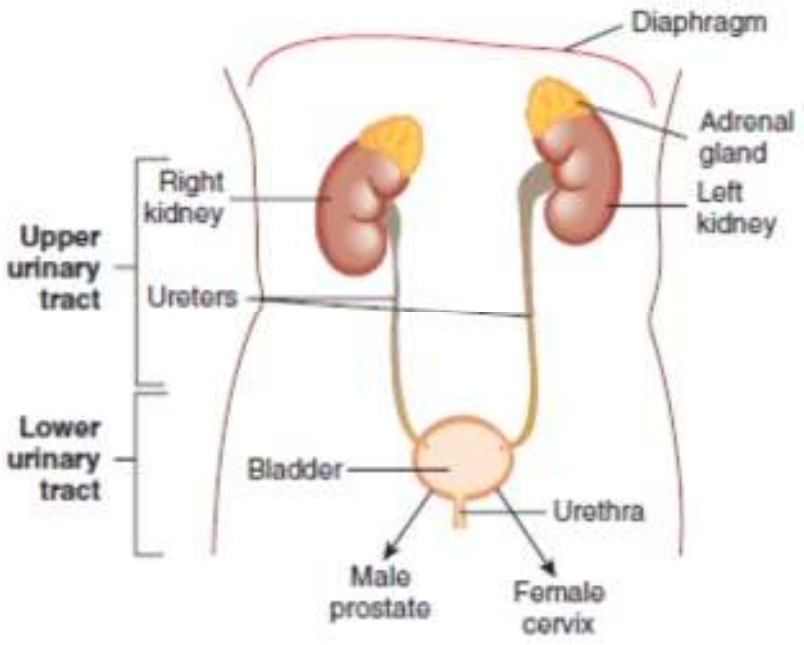


Fig 1: **Anatomy of the urinary tract**[1]

The urinary bladder is situated within the pelvis behind the pubic bones. It stores urine and in the adult has a maximum capacity of about 500 ml. The empty bladder is pyramidal, having an apex, a base, and a superior and two inferolateral surfaces; it also has a neck which lies just below the level of the upper border of the symphysis pubis. The **base,** or **posterior surface** of the bladder, faces posteriorly and is triangular. The superolateral angles are joined by the ureters, and the inferior angle gives rise to the urethra. The ureters pierce the bladder wall obliquely, and this provides a valve like action, which prevents a reverse flow of urine toward the kidneys as the bladder fills.

The urethra in males longer than females and measures 20 cm. It is divided into three parts: prostatic urethra, membranous urethra and penile urethra. Whereas, the urethra in females about 3.8 cm long.

Anatomically, the urinary tract is divided as upper and lower urinary tract: lower implies the bladder and urethra, and the upper urinary tract implies ureters and kidneys.

TYPES OF URINARY TRACT INFECTION[1,10]

Any inflammatory response of the urothelium to bacterial invasion is UTI. It is usually associated with bacteriuria and pyuria.

a) Based on the presumed site of origin, UTI can range from cystitis, pyelonephritis to urethritis.
b) Based on the anatomic location of the infection, UTI is classified as:
    i. Upper UTI - It affect the ureters (ureteritis) or the renal parenchyma (pyelonephritis).

ii. Lower UTI- It may affect the urethra (urethritis), the bladder (cystitis), or the prostate in males (prostatitis).

c) Based on the health of the host and anatomic/functional status of urinary tract, UTI can be:

i. Uncomplicated UTI- it occurs when the urinary tract is structurally and functionally normal and the host is not compromised.

ii. Complicated UTI- Here the urinary tract is structurally or functionally abnormal, the host is compromised and/or the bacteria have increased virulence or antimicrobial resistance.

CATHETER ASSOCIATED URINARY TRACT INFECTION (CAUTI) AND ITS DIAGNOSIS [6,12,13]

An indwelling catheter, also known as Foley's catheter, is a drainage tube that is inserted into the urinary bladder through the urethra, is left in place, and is connected to a drainage bag (including leg bags. Condom or straight in-and-out catheters, nephrostomy tubes or suprapubic catheters are not included. The indwelling urinary catheters are made up of tuber latex or silicon. They are essential components of modern medical care and are used in acute care setting and extended care facilities. Unfortunately, they significantly increases the risk of iatrogenic infection

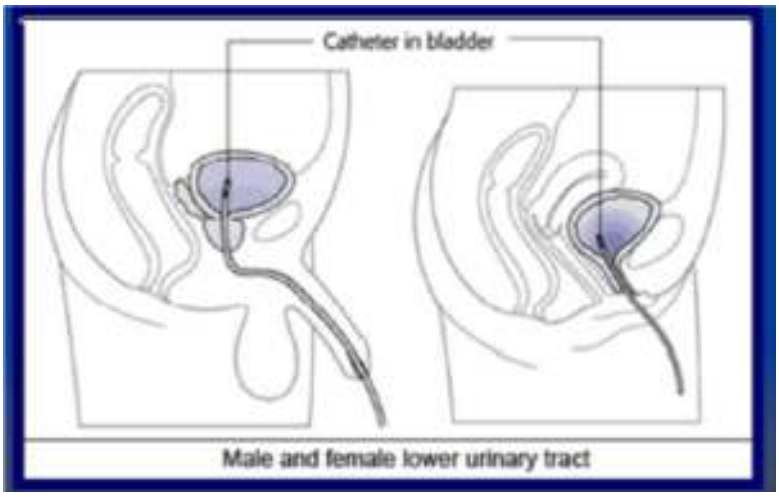


Fig 2: **Foley's catheter**[13]

Catheter associated urinary tract infection as a UTI where an indwelling urinary catheter was in place for >2 calendar days on the date of event, with day of device placement being Day 1, *and* an indwelling urinary catheter was in place on the date of event or the day before. If an indwelling urinary catheter was in place for > 2 calendar days and then removed, the UTI criteria must be fully met on the day of discontinuation or the next day.

If in a patient develops UTI within 48 hours of discharge from a location, The infection is attributed to the transferring location or facility. This is known as the "transfer rule".

In case the Foley's catheter is discontinued and reinserted and one full day passes between the two events, then the day count should be started anew. Otherwise the day count continues from the previous catheter.

It is of 2 types: Symptomatic CAUTI & Asymptomatic bacteremic CAUTI. UTI has more typically been assumed to imply symptomatic disease that warrants antimicrobial therapy.

- **Symptomatic CAUTI:** [6] It is diagnosed as:

1. Patient had an indwelling urinary catheter >2 days & **at the time** of specimen collection(day of event)

and

- at least 1 of the following signs or symptoms:
  - ✓ fever (>38°C);
  - ✓ suprapubic tenderness;
  - ✓ costovertebral angle pain or tenderness (With no other recognized cause)

and

- a positive urine culture of **≥10^5 CFU/ml** and with no more than 2 species of microorganisms.

2. Patient had an indwelling urinary catheter for >2 days & had it **removed** the day of or the day before ,

and

- at least 1 of the following signs or symptoms:
  - ✓ fever (>38°C);
  - ✓ suprapubic tenderness;
  - ✓ costovertebral angle pain or tenderness (With no other recognized cause)
  - ✓ urgency,
  - ✓ frequency,
  - ✓ dysuria,

and

- a positive urine culture of **≥10^5 CFU/ml** and with no more than 2 species of microorganisms.

3. Patient had an indwelling urinary catheter >2 days & **at the time** of specimen collection

and

- at least 1 of the following signs or symptoms:
  - ✓ fever (>38°C);
  - ✓ suprapubic tenderness;
  - ✓ costovertebral angle pain or tenderness (With no other recognized cause)

and

- a positive urine culture of **≥10^3 & ≤ 10^5 CFU/ml** and with no more than 2 species of microorganisms.

and

- at least one of the following findings:
  i. Positive dipstick test for leukocyte esterase &/nitrite
  ii. Pyuria (urine specimen with ≥10 white blood cells [WBC]/mm^3 of unspun urine or >5 WBC/high power field of spun urine)
  iii. Microorganisms seen on Gram's stain of unspun urine

4. Patient had an indwelling urinary catheter for >2 days & had it **removed** the day of or the day before ,

and

- at least 1 of the following signs or symptoms:
  - ✓ fever (>38°C);

- ✓ suprapubic tenderness;
- ✓ costovertebral angle pain or tenderness (With no other recognized cause)
- ✓ urgency,
- ✓ frequency,
- ✓ dysuria

and

- a positive urine culture of **$\geq 10^3$ & $\leq 10^5$** CFU/ml and with no more than 2 species of microorganisms.

and

- at least one of the following findings:
    i. Positive dipstick test for leukocyte esterase &/nitrite
    ii. Pyuria (urine specimen with ≥10 white blood cells [WBC]/mm3 of unspun urine or >5 WBC/high power field of spun urine)
    iii. Microorganisms seen on Gram's stain of unspun urine

EPIDEMIOLOGY OF CATHETER ASSOCIATED URINARY TRACT INFECTION

Across the world, over 100 million urinary catheters are being used annually. This turns out to be almost 200 being used every single minute [14,15].

Up to 25% of hospitalized patients are catheterised. Though it is a necessary intervention, indwelling catheters are a leading cause of nosocomial infection[16]. Various studies have shown that 75 to 80% of all nosocomial urinary tract infections occur following catheter insertion.[17]

Most CAUTIs are benign, in 30-40% of patients, a systemic complication like gram negative bacteremia can develop. So, to decrease the expenses secondary to infection and morbidity and mortality rate, prevention of CAUTI and its complications is very essential.[18]

CAUTIs in hospitalized patients are most likely to be caused by Enterobacteriaceae such as *Escherichia coli, Klebsiella* spp. and *Proteus* species, *Staphylococcus* species, *Pseudomonas aeruginosa* and *Enterococcus* species.[1]

The organisms causing urinary tract infections vary from one geographical area to another. The increased prevalence of UTIs caused by some highly resistant pathogens and the changing resistance pattern of isolates from time to time is due to catheterisation.[16]

In ICUs, nosocomial infections are common due to frequent use of wide-spectrum antibiotics, presence of comorbid conditions and increased use of invasive interventions and. In developing countries, the rate of healthcare associated infections and bacterial resistance are 3 to 5 times higher than international standards.[19]

RESIDENT MICROORGANISMS OF URINARY TRACT [1, 20, 21]

The normal commensals of the urethra are lactobacilli, corynebacteria, and coagulase-negative staphylococci. All areas of the urinary tract above the urethra in a healthy human are sterile. The resident microflora include:

- Coagulase-negative staphylococci (excluding *S. saprophyticus*)
- Viridans and non-hemolytic streptococci
- Lactobacilli

- Diphtheroids (*Corynebacterium* spp.)
- Nonpathogenic (saprobic) *Neisseria* spp.
- Anaerobic gram-negative bacilli
- Anaerobic cocci
- *Propionibacterium* spp.
- Commensal *Mycobacterium* spp.
- Commensal *Mycoplasma* spp.

ETIOLOGICAL AGENTS OF CAUTI[1,21]

The presence of a foreign body in the urinary tract increases the chances of CAUTI. The pathogens are acquired mainly from the hospital niche such as *E. coli, Klebsiella* spp., *Proteus* spp., staphylococci, other Enterobacteriaceae, *Pseudomonas aeruginosa*, enterococci, and *Candida* spp.

PATHOGENESIS [1,7,22]

The Foley's catheter interferes with the natural defences of the urinary tract.

Routes of infection:

Urine is typically sterile. There are three main routes for the entry of organisms into the urinary tract: ascending, haematogenous and lymphatic. CAUTI is mainly an ascending infection. Though innate mechanisms prevent infection, these pathogens can cause infection because they colonise urinary catheter and/or mucosal site, evade host defences and replicate and damage the host cells. Patients become colonised with microorganisms endemic to the hospital soon after hospitalisation which carry resistant markers. These organisms colonise different areas of the patient such as skin,

gastrointestinal tract and mucous membrane. Organisms may cause CAUTI in one of two ways:

a. **Extraluminal ascending infection**- It can occur either during the time of indwelling catheter insertion, or later on from the perineal area. The external surface of the Foley's catheter is coated by a thin mucous film in which the organisms can move upwards by capillary action. This extraluminal route is responsible for 66% of CAUTI.

b. **Intraluminal infection** – Here, the organisms enter the lumen of the catheter due to manipulation of the catheter system. Either the closed drainage system is not maintained or the urine in the collecting bag gets contaminated. It is responsible for 34% of the cases.

These organisms multiply and then ascend through ureters to the kidneys once they reach the bladder.

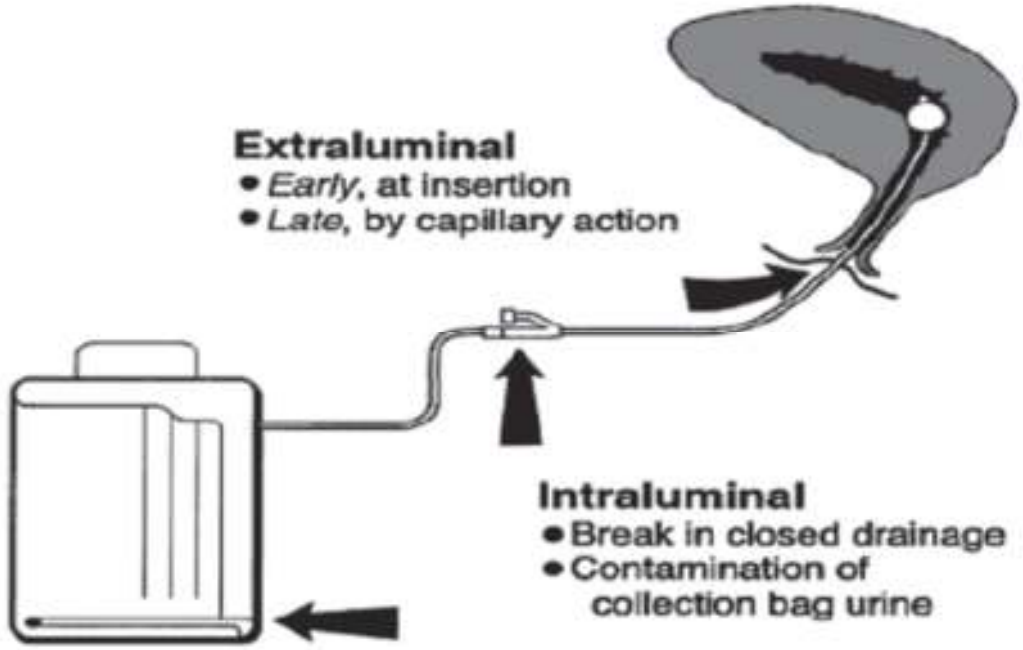


Fig 3: **Routes of infection of CAUTI** [22]

Host parasite relationship:[1,7]

Indwelling pathogens favour the colonisation of uropathogens due to the following factors:

a) Enhancing microbial adhesion- The bacteria recognise the surface of the catheters by adhesins and attach to the host cell binding receptors.
b) The protective uroepithelial mucosa get damaged while inserting catheter, thus exposing new binding sites for adhesins.
c) Indwelling catheter disrupts the normal host mechanical defences. Residual urine due to overdistension of bladder and incomplete voiding, favours microbial growth.

Fewer virulence factors are required by organisms which cause CAUTI.

The various **bacterial virulence factors** are:

1. **Bacterial adhesins**- These help in attaching to the host cell receptor. The adhesins overcome the electrostatic repulsion present between bacterial cell membrane and surfaces, thus allowing intimate interactions. During the infection, these factors are expressed differentially. They recognise specific host cell surfaces, cell types and extracellular membrane proteins and also evade the host immune response. Gram negative uropathogens have adhesinson the tip of fimbriae or pili and non-fimbrial adhesins.

2. **Biofilm formation**- They start to change phenotypically and produce exopolysaccharide after attaching to the surface of catheter and urothelium. These entrap and protect these pathogens. They multiply and form microcolonies and mature into biofilms.

The advantages of biofilm are:

a) There is exchange of genetic material among the organisms within the biofilm which causes acquisition and spread of antibiotic resistant genes and other characteristics.

b) It protects the uropathogens from antimicrobials and host immune response.

c) The biofilm aggregates are sheared and daughter cell from actively growing cells are shed. This causes seeding and infection of bladder and catheter.

3. Motility – Motility of the bacteria mediated by flagella and type IV pilus helps in spreading the infection from the initial colonisation site to the urinary tract.

4. Toxins- The organisms adapt to the urinary tract environment on colonising on the catheter and urothelium. Due to degradative enzymes and toxins produced by them tissues are broken down and nutrients released.

5. Iron acquiring systems- Various iron acquisition systems such as ferric and ferrous ion transport systems, heme transporters, and siderophore iron uptake system are developed by the organisms.

6. Urea utilisation- Many bacteria utilise urea as a nitrogen source present in human urine. Hydrolysis of urea alkalinises the urine and cause precipitation of polyvalent ions. These get enmeshed and form crystalline biofilms which protect the biofilm associated bacteria from antimicrobial agents.

7. Evasion of host immune response-

a) Production of capsule

b) Immunoglobulin A proteases

c) Lipopolysaccharides-They help in resisting antimicrobial peptides and complement-mediated lysis.

Risk factors :[22,23]

Various risk factors are attributed for the causation of CAUTI. These include:

1. **Duration of catheterisation** – Foley's catheter can be short-term (*in situ* less than 28 days), or long-term (*in situ* greater than 28 days). The risk of acquiring an infection increases by 5% each day.
2. **Female gender** - The anatomy of the female urethra is significantly important for the pathogenesis of UTIs as it is relatively short compared with the male urethra and also lies in close proximity to the warm, moist, perirectal region, which has abundant microorganisms. So, in females, the pathogens can reach the bladder more easily. For men, the incidence of urinary tract infections increases after the age of 60 because the enlarged prostate causes incomplete voiding of urine.
3. **Diabetes mellitus**
4. **Underlying neurological disease**
5. **Respiratory diseases such as COPD**
6. **Urological/ nephrological diseases**
7. **Steroid use and other immunocompromised conditions**

INDICATIONS FOR CATHETERISATION [22]

Some of these include relieving urinary retention, assist in achieving patient immobilisation in case of fractures, monitor urinary output in critically ill patients,

instillation of drugs or during urology investigations and for patient comfort during end of life care.

COMPLICATIONS OF FOLEY'S CATHETERS [23,24]

The presence of indwelling catheter leads to various infections such as cystitis, pyelonephritis, secondary bacteremia/sepsis or late onset sequellae, e.g. metastatic osteomyelitis and meningitis.

As a result, hospital stay is prolonged and there is emergence of multidrug resistant organisms, thereby increasing morbidity, mortality and health cost.

LABORATORY DIAGNOSIS [1,8,21,25]

1. **Specimen collection**- Strict aseptic technique should be followed while collecting urine specimen from patients with indwelling catheters. Gloves should be worn by healthcare workers. The sample should be collected ideally from the sampling port. If sampling port is not present, then the catheter tubing should be clamped off above the port and the wall of the tubing cleaned with 70% ethanol. The urine is then aspirated using a needle and syringe. Minimum 3 ml of the urine sample should be collected from Foley's catheter. There should not be any disruption of the closed drainage system. Specimens obtained from the collection bag are inappropriate, because organisms can multiply there, obscuring the true relative numbers.

2. **Specimen transport**- Urine must be immediately transported to the lab within 2 hours of collection or must be immediately refrigerated or preserved.

When there is a delay in the transport of urine sample for more than 2 hours, boric acid (0.1g/10 ml of urine) is used as a preservative. Such specimens need not be

refrigerated. At this concentration of boric acid, bacteria remain viable without multiplying for 48 hours, however it may inhibit some *Enterococci* and *Pseudomonas spp.*

Urine must not be preserved with a bactericidal chemical such as thymol, bleach, hydrochloric acid, acetic acid or chloroform.

For patients from whom colony counts of organisms of less than 100,000/mL might be clinically significant, plating within 2 hours of collection is recommended. The kits provide a convenient method for preserving and transporting urine from remote areas where refrigeration is not practical.

3. **Specimen processing**

a. Screening procedures- As a major portion of urine specimens may contain no etiologic agents of infection or contain only contaminants, procedures have been developed to identify quickly those urine specimens that will be negative on culture and circumvent excessive use of media, technologist time, and the overnight incubation period are discussed in this section. A reliable screening test for the presence or absence of bacteriuria provides physicians important same-day information that a conventional urine culture may take a day or longer to provide. Many screening methods have been advocated for use in detecting bacteriuria and/or pyuria. These include microscopic methods, colorimetric filtration, bioluminescence, electrical impedance, enzymatic methods, photometric detection of growth, and enzyme immunoassay.

Some of these are:

i. **Gram stain –** A Gram stain of urine is an easy, inexpensive means which provides immediate information about the infecting organism and thereby guides empiric therapy. The performance characteristics of the urine Gram stain are not well defined because different criteria have been used to define a positive result (1 or 5 bacteria per OIF). Using either 1 or 5 bacteria/OIF has a sensitivity of 96% and 95%, respectively, and a specificity of 91% when correlated with significant bacteriuria ($>10^5$ CFU/mL).

The Gram stain should not be used for detecting polymorhonuclear neutrophils (PMNs) in urine because leukocytes deteriorate quickly when the urine is not fresh or not adequately preserved.

ii. **Pyuria-** The presence of pus cells in urine signifies inflammation. These can be detected and enumerated in uncentrifuged or centrifuged specimens. In urine sediments, white blood cells (WBC) are usually reported as:

Few: Up to 10 WBCs/HPF (high power field, i.e. using 40x objective)

Moderate number: 11–40/HPF

Many: More than 40 WBC/HPF

A few pus cells are normally excreted in urine. Pyuria is significant when more than 10 WBC/µl of urine are present.

iii. **Nitrate reductase test-** This detects the presence of nitrites in the urine. The pathogens belonging to the family Enterobacteriaceae reduce nitrate to nitrite by the enzyme nitrate reductase. This is detected by the Greiss test or a nitrite reagent strip test, provided the organisms are present in sufficient concentration. The test is negative when the organisms are few or the pathogen are *Enterococcus faecalis,*

Pseudomonas species, and Candida spp. which do not reduce nitrate to nitrite. A positive nitrite test shows development of pink- red colour.

iv. Leucocyte esterase test- The evidence of a host response to infection is the presence of PMNs in the urine. Leukocyte esterase enzyme is specific for PMNs. This test detects the enzyme from both active and lysed WBCs. The nitrate reductase and leukocyte esterase tests have been incorporated into a reagent paper strip.

False negative results can occur when the urine contains boric acid or excessive amount of protein (>500 gm/100ml) or glucose (> 2 gm/100ml). Unreliable results can also occur when urine contains nitrofurantoin of gentamicin.

b. <u>Urine culture-</u> Urine culture can be performed by semi-quantitative method, quantitative method and automated system

❖ **<u>Semi-quantitative method-</u>**

✓ **Standard loop method-**The urine should be mixed thoroughly before plating. Inoculation of the plates is done using disposable sterile plastic tips calibrated to deliver a constant amount or calibrated loop which delivers either 0.1 or 0.01 ml of urine.

The calibrated loop that delivers 0.01 mL of urine is recommended to detect lower numbers of organisms in certain specimens such as samples from Foley's catheters, nephrostomies, ileal conduits, and suprapubic aspirates should be plated with the larger calibrated loop. The media used are either Cysteine lactose electrolyte deficient (CLED) or 5 % sheep blood agar and Mac Conkey agar. The inoculated plates are incubated for at least 24 hours at 35° to 37° C in air. Colonies are counted on each plate. The number of CFUs is multiplied by 1000 (if a 0.001-mL loop was used) or by

100 (if a 0.01-mL loop was used) to determine the number of microorganisms per millilitre in the original specimen. Plates with no growth are incubated for total of 48 hours before discarding them.

✓ **Filter paper method**- This method uses a standard 6mm wide strip of blotting paper or filter paper. It is bent into L-shape with a 12mm long foot and sterilised at 160 °C for one hour. The whole of the angulated end and foot is dipped in the uncentrifuged urine sample and then withdrawn and waited for few seconds so that the excess of urine is absorbed on the filter paper. The foot of the paper is then pressed on the culture medium so that the whole area of the foot makes contact with the medium. The strip is removed and discarded in the disinfectant. The plate is read after overnight incubation for the number of colonies. Up to 50 colonies can be counted and heavier growths are designated as confluent (++) or semi-confluent (+). A count of 25 colonies of bacilli and 30 colonies of cocci corresponds to 10^5 bacteria/ml count.

✓ **Dip slide method-** The dip slide is a small plastic tray which contains a layer of the appropriate culture medium. The dip slide is withdrawn and briefly immersed in the urine container. On removing, any excess of the urine is removed against the wall of the urine container and the dip slide is then replaced in its container. It is incubated overnight at 37° C. the count of viable bacteria is estimated from the number of colonies or the pattern of confluent or semi-confluent growth. Commercial suppliers provide charts showing representative numbers and patterns. By comparing with it, the viable count can be read.

❖ **Quantitative method** [26]- This includes pour plate method and Miles & Misra method. These methods measure CFU/ml which gives the count of viable bacterial or fungal cells.

In Miles and Misra method, nutrient agar plates are used which are divided into four. Three serial dilutions - one in ten, one in one-hundred and one in one-thousand are made of the sample and two drops (20 µl each) of the original sample and the dilutions are inoculated into the quadrants of each plate. The plates are incubated overnight and inspected for growth. Separate colonies that are countable will be seen in one of the four quadrants.

An average of the counts on the five plates is made. The CFU/ml is calculated using the formula:

cfu/ml = (no. of colonies x dilution factor) / volume of culture plate

❖ **Automated system**[27]-Sidecar, Alfred 60AST and HB&L are the first automated systems for the rapid urine culture. These have high sensitivity and specificity. The technology based on light scattering is used. They can monitor the bacterial replication activity and provide real time growth curves. After only 45 minutes of incubation, strong positive samples get flagged. Only live bacteria are detected in this method. The interference from erythrocytes, leucocytes, dead cells and salts present in the sample are eliminated during the initial zero reading

PREVENTION OF CAUTI[7]

The various measure to prevent CAUTI include:

1. **Avoid unnecessary catheterization**- The indwelling catheter use should be limited to those patients who really require catheterisation. If required for more than a week, the urinary catheters should be changed every 8 to 10 days to reduce the risk of infection. The drainage bags should be emptied at a minimum of every 4 to 6 hours to avoid bacteria entering the catheter lumen.

2. **Select alternative catheterization procedures**- Alternative methods of catheterisation such as condom and suprapubic catheters and intermittent catheterisation should be considered.

3. **Maintenance of a closed drainage system and proper use of catheter**- Proper aseptic technique should be used for the insertion the urinary catheter. A closed catheter system must be maintained. The catheter and the drainage tube should not be disconnected at any time. The correct size of the catheter should be used. Smaller catheters (14 French and 16 French) and 5 ml balloons are preferred because the larger catheters tend to increase the amount of residual urine and cause reinoculation of the bladder. There are chances of blockage of paraurethral glands, urethral irritation and erosion.

4. **Eliminate bacterial colonization of the meatus**- Proper meatal care should be done with daily cleansing with soap and water or with povidone- iodine solution followed by povidone-iodine ointment. It should be done at least twice daily.

5. **Development of new biomaterials for catheter**- Hydrophobicity of the organisms and biocompatible surface of the catheter determines bacterial adhesion. Biomaterial such as silicone, polyurerhrane, composite biomaterial or hydrogen coated

material have been studied for preventing colonisation of catheter and biofilm formation.

6. **Use of antimicrobial agents on catheter materials**- Preventive measures such as application of antimicrobial solutions, antiseptics and lubricants on the catheter surface and the addition of antimicrobial agents in the collection bag have been studied but have not proven any benefit.

7. **Use of probiotics**- Adding non-pathogenic strains into the bladder can prevent symptomatic infection by bacterial interference and hindering uropathogen colonisation.

8. **Newer technologies**- Newer approaches such as the sensing of bacterial encrustation, use of biofilm inhibitors, development of hydrophilic and nutrient-scavenging biomaterials, and use of low-energy surface acoustic waves have been developed.

Catheter bundle care is followed to reduce the incidence of catheter associated urinary tract infection [28] . These include:

- ✓ Appropriateness of the indication for catheterisation
- ✓ Monitoring of maintenance of closed drainage system
- ✓ Routine daily meatal hygiene
- ✓ Routine emptying of drainage bags
- ✓ Use of proper hand hygiene, gloves and apron before each catheter care procedure.

TREATMENT[7]

Asymptomatic bacteriuria is inevitable in catheterised patients. So, its treatment is usually not recommended. Also, it may cause selection of resistant strains and emergence of multidrug resistant organisms.

Treatment of Symptomatic CAUTI is challenging as these are usually polymicrobial in nature especially in long-term catheterised patients and due to the presence of antimicrobial resistance and biofilm formation. Most of the patients are treated with antimicrobials for 10-14 days usually with parenteral antimicrobials. In case of monomicrobial infection and short term catheterisation, treatment consists of single drug therapy. Catheterised patients who are seriously ill and those with polymicrobial infection and long-term catheterisation require two drug regime. Once antimicrobial therapy has been administered, the indication of clearance of infection is resolution of symptoms and not the absence of bacteriuria.

MATERIALS AND METHODS

ETHICAL CONSIDERATION:

Approval of the Institutional Ethics Committee was obtained before starting the study. Informed written consent was taken from all the patients included in the study.

STUDY SETTING:

Study Design: Cross-sectional study

Study duration: One year- October 2014 to September 2015

Sample size: 100 patients

Study Group: Patients admitted to medical ICU and put on Foley's catheter

Place of study: The study was conducted at the Institute of Microbiology, Madras Medical College in association with Medical ICU, Rajiv Gandhi Government General Hospital, Chennai.

INCLUSION CRITERIA:

- Age ≥ 18years
- Patient admitted to IMCU and put on Foley's catheter.

EXCLUSION CRITERIA:

- Patients <18 years of age
- Patients catheterised prior to admission in ICU

- Those patients, whose Foley's catheter were **removed** or who were **discharged/expired** before 3rd day of catheterization
- Those who were confirmed to have UTI on Day 1
- Pregnancy

COLLECTION OF DATA[8]:

Data were collected from the patients included in the study using a preformed structured questionnaire. Details such as name, age, sex, address, IP no., date of admission, clinical data like presenting complaints, personal history, past medical history, high risk factors, immunocompromised status, physical examination findings and details of clinical diagnosis were collected. Indication for Foley's catheter was noted. Daily examination of the patients were done to look for any evidence of urinary tract infection such as fever, suprapubic tenderness and costovertebral angle tenderness. Catheter care in the form of daily meatal care by betadine or soap water and maintenance of closed drainage were frequently monitored. The patients were followed till they developed bacteriuria or discharged, expired or catheter was removed. The date of catheter removal and duration of catheterization was noted. Patients who were shifted to different ward were followed for up to 48 hrs for the developments of symptoms of CAUTI.

COLLECTION OF URINE SAMPLE[1,8]:

Urine specimens were collected aseptically. The Foley's catheter was first clamped above the bifurcation and then disinfected with spirit. Using a sterile syringe

24-gauge needle, approximately (minimum) **3ml** of urine was taken as a sample from above the area clamped, in a flat bottomed universal container.

The samples were taken to laboratory within **1 hour** of collection. Day **1** sample was taken to rule out prior presence of UTI. The samples were repeated on **3rd, 5th, 7th, 10th, 14th** day and then every weekly until catheter removal, or patient developed bacteriuria, or until discharge/death of the patient.

SAMPLE PROCESSING [1,21]:

1. **Direct Gram's stain of uncentrifuged urine-** A loopful of urine was taken on a clean, sterile glass slide, smear prepared and Gram stained. It was air dried and observed under oil immersion lens for the presence of bacteria or candida. Presence of one microorganism per oil immersion field indicates bacterial count of $>10^5$/ml of urine.
2. **Dipstick for leukocyte esterase and/or nitrite**- DIRUI H-10 urinalysis strips [29] were used for detection of nitrites and leucocyte esterase. The test was done on uncentrifuged urine. The reagent area of the strip was immersed in the urine and removed quickly. Excess of urine was removed by holding the edge of the strip against the rim of the container. The result on the strip was compared with the colour chart on the bottle label.
3. **Wet mount for WBC** – The urine sample was centrifuged at 3000rpm for 3-5 minutes. The supernatant was discarded and a drop of sediment was placed on the glass slide and a cover slip placed to prepare a wet mount. The number of pus cells / high

power field was counted under 40 x objective. More than 5 WBC/hpf was considered significant for diagnosing CAUTI.

4. **Urine culture-** The specimens were cultured by semi-quantitative method using Mac Conkey Agar and Blood Agar as culture medium.

   i. **Inoculation of the medium** [1] - The catheterized urine specimen was inoculated by calibrated loop method. Before inoculation, urine was mixed thoroughly and the top of the container then removed. A calibrated loop (4 mm loop) that delivers 0.01 mL volume of urine was used. It was inserted vertically into the urine in the cup and taken out vertically. It was then touched to the centre of the plate and the inoculum was spread in a line along the diameter of the plate. Without flaming or re-entering urine, the loop was drawn across the entire plate, crossing the first inoculum streak several times perpendicular to the first streak. 5% Sheep blood agar plate and Mac Conkey agar plate were streaked by this method. They were incubated at 37°C for minimum 24 hours.

   ii. **Interpretation of the culture** – The plates were read after 24 hours of incubation for any growth. The number of colonies were counted. The number of CFUs was multiplied by 1000 to determine the number of microorganisms per milliliter in the original specimen. The plates with no growth or tiny colonies were incubated for an additional 24 hours before discarding.

5. **Identification of isolates** [30,31]- The isolates were identified based on colony morphology in 5% sheep blood agar and Mac Conkey Agar and Gram stain of the smear made from the colonies.

i. <u>Identification of Gram negative Bacilli</u>- Those colonies which showed GNB were further subjected to preliminary tests such as motility by hanging drop method, catalase and oxidase tests. The GNBs that were catalase positive, oxidase negative were suspected to belong to the family *Enterobacteriaceae*.

The GNBs which were catalase positive and oxidase positive were suspected of being non-fermenters. All these organisms were further speciated based on standard biochemical tests. These included nitrate reductase test, 1% glucose Oxidative/ fermentative test, indole production, Methly red test, Voges Proskauer test, citrate utilization, triple sugar iron test, urease production, phenyl alanine deaminase test , decarboxylation of lysine, ornithine and of arginine in Moeller's decarboxylation media and a series of basic sugar fermentation tests – glucose, lactose, sucrose, maltose and mannitol.

The Pseudomonads were speciated based on pigment production and additional biochemical tests such as gelatinase test, starch hydrolysis and xylose fermentation

ii. <u>Identification of Gram positive cocci</u>- Those colonies morphologically resembling Staphylococci were further identified by catalase test, modified oxidase test, tube coagulase and slide coagulase test. The biochemical tests were nitrate reductase, methyl red, Voges Proskauer, urease production and sugar fermentation tests.

Suspected Enterococci colonies were identified and speciated by catalase test, bile esculin agar, heat test, Voges Proskauer test, arginine hydrolysis and fermentation of sugars- arabinose, raffinose, sorbitol, mannitol and sucrose.

iii. <u>Identification of Candida species</u>- Based on colony morphology on 5% sheep blood agar and no growth on Mac Conkey agar, the colonies were suspected to belong to Candida species. Gram stained smear showed Gram positive budding yeast cell with pseudohypahae. Candida was further speciated based on germ tube test, Dalmau plate culture method, Chrom agar, sugar fermentation and sugar assimilation tests.

a) <u>Germ tube test</u>[32,33]- 0.5 ml of human serum was taken in a sterile test tube and an isolated colony of yeast was emulsified. It was incubated at 37˚C for two hours. A drop of this suspension was placed on a glass slide, a cover slip applied over it and observed under high power for the presence of germ tube formation.

Positive - *Candida albicans / Candida dubliniensis*

Negative – *Non-albicans Candida spp*

b) <u>Dalmau plate culture technique/ cornmeal tween 80 agar</u>[34,35]-An isolated yeast colony was taken from the primary culture using a straight wire. It was inoculated onto cornmeal agar with Tween 80 plate by making 2-3 parallel lines 3.5-4cm long and 1-2 cm apart. The streak lines were then covered with sterile coverslip and incubated at 25˚C for at least 48 hours. It was examined under low power and then under high power objective at the margin of the coverslip for the presence of hyphae, blastoconidia and chlamydospores.

Table 1: Morphology on cornmeal tween 80 agar

Candida albicans	Pseudohyphae with clusters of round blastoconidia at the septa; large thick-walled, **single,** terminal chlamydospores.
C.dublinensis	Pseudohyphae with some true hyphae; clusters of round blastoconidia at the septa; pairs or **clusters** of large thick-walled, terminal chlamydospores
C.tropicalis	Abundant slender long pseudohyphae**, pine forest arrangement**, blastoconidia singly or in very small groups along pseudohyphae, few tear drop shaped chlamydospores may be present.
C.parapsilosis	Single or small clusters of blastoconidia; **crooked or curved** appearance of short pseudohyphae; presence of large hyphal elements called **"giant cells"**.
C.krusei	Pseudohyphae with elongated blastoconidia giving **"match-stick appearance"**
C.kefyr	Pseudohyphae with elongated blastoconidia that lie parallel giving **"log in stream appearance"**.
C.guilliermondi	Clusters of yeast cells; relatively few short pseudohyphae; **small groups** of blastoconidia at the septa; true hyphae not produced
C.glabrata	Only small oval yeast cells with single terminal budding; no pseudohyphae.
C.lusitaniae	Short, distinctly curved pseudohyphae with occasional blastoconidia at septa.

c) Candida CHROM agar[32,35,36]

Candida spp. were sub-cultured onto Sabourauds Dextrose agar plate and was then streaked onto Chrom agar plate. The plates were incubated for 48 hours at 37°C. The colour and morphology of the colonies were noted.

Table 2: Appearance on Chrom agar

Species	**Colour**
Candida albicans	Green
C.dublinensis	Light green
C.tropicalis	Steel blue
C.parapsilosis	White to pale pink colonies
C.krusei	Large , flat, spreading pale pink colonies with matt surface
C.kefyr	Pink to lavender
C.guilliermondi	Pink to purple
C.glabarata	Smooth glossy cream to pink colonies
C.lusitaniae	Pink grey purple

d) Sugar fermentation tests[32,33,35]- Candida spp. were subcultured in sugar free media at 37°C for 24-48 hours. Then 0.2 ml suspension were added to 2% sugar fermentation media containing Andrade indicator. They were incubated at 30 °C for 48 to 72 hours. Fermentation was indicated by acid and gas production. The sugars tested were glucose, maltose, sucrose and lactose.

Table 3: Fermentation Reaction

Species	Glucose	Maltose	Sucrose	Lactose
C. albicans	AG	AG	-	-
C. tropicalis	AG	AG	AG	-
C. kefyr	AG	AG	AG	-
C. parapsilosis	AG	-	-	-
C. krusei	AG	-	-	-
C. glabrata	AG	-	-	-
C.guilliermondi	AG	-	AG	-
C. lusitaniae	AG	AG	-	-

A: acid production; G: gas production

e) Sugar assimilation test[32,33,35] – The test organism was sub-cultured on non-nutrient agar. 4-5 colonies of the yeast were emulsified in 2 ml of normal saline. To the yeast suspension, 15 ml of yeast nitrogen agar base was added and mixed well and allowed to set at room temperature in petri-plate. Carbohydrate discs -glucose, maltose, sucrose, lactose, cellibiose, galactose, trehalose, raffinose, xylose, inositol and dulcitol were placed on the agar and incubated for 24-48 hours at 25°C. The assimilation of the particular carbohydrate by the yeast is indicated by the growth around the discs. The pattern of assimilation was noted.

Table 4: Carbohydrate Assimilation

Species / Carbohydrates	*Candida albicans*	*C. dublinensis*	*C. tropicalis*	*C. parapsilosis*	*C. krusei*	*C. kefyr*	*C. guilliermondi*	*C. glabrata*
Glu	+	+	+	+	+	+	+	+
Mal	+	+	+	+	-	-	+	-
Suc	V	V	+	+	-	+	+	-
Lac	-	-	-	-	-	+	-	-
Cel	-	-	+	-	-	+	+	-
Gal	+	+	+	+	-	+	+	-
Tre	+	-	+	+	-	-	+	+
Raff	-	-	-	-	-	+	+	-
Xyl	+	-	+	+	-	+	+	-
Ino	-	-	-	-	-	-	-	-
Dul	-	-	-	-	-	-	+	-

+ positive, - negative, V variable

ANTIMICROBIAL SUSCEPTIBILITY TEST

A. ANTIBACTERIAL SENSITIVITY TEST

Kirby-Bauer disc diffusion method was used for antimicrobial sensitivity test and MIC was done for drugs meropenem and vancomycin.

1) Kirby Bauer Disk Difffusion Method[37]

Preparation of inoculum and Application of discs:-

1. With a sterile bacteriological loop, 3- 4 well isolated and identical colonies were emulsified in 3-4ml of sterile peptone water.
2. They were incubated for two hours at 37ºC and the density of the suspension was matched to 0.5 McFarland standard.
3. A sterile cotton-wool swab was dipped into the suspension within 15 minutes and excess removed by rotating the swab against the side of the test tube.
4. It was streaked evenly on to the entire surface of the Mueller Hinton Agar plate. Streaking was done in three different directions, rotating the plate approximately 60°C to obtain a lawn culture and ensure even distribution.
5. The inoculated plate was allowed to dry for 3 to 5 minutes and the antibiotic discs were placed, maximum of six on each plate.
6. With each batch of tests, control strains were included as per the CLSI guidelines.

Reading the sensitivity pattern:

After 16-18 hours of incubation at 37°C, the diameter of zones of inhibition were measured in mm (as judged by the unaided eye), including the diameter of the disk.

Panel of drugs were used for antimicrobial sensitivity testing:

Table 5: Gram negative bacilli (Enterobacteriaceae, Pseudomonas)

Anibiotics	Disc content	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Ampicillin *#	10 µg	≥17	14-16	≤13
Amikacin	30 µg	≥17	15-16	≤14
Gentamicin	10 µg	≥15	13-14	≤12
Norfloxacin	10 µg	≥17	13-16	≤12
Nitrofurantoin#	300 µg	≥17	13-16	≤14
Trimethoprim-sulfamethoxazole (Cotrimoxazole) #	1.25/ 23.75 µg	≥16	11-15	≤10
Cefotaxime#	30 µg	≥26	23-25	≤22
Ceftazidime *Enterobacteriaceae*	30 µg	≥21	16-20	≤17
Pseudomonas aeruginosa		≥18	15-17	≤14
Tetracycline#	30 µg	≥15	12-14	≤11
Piperacillin- Tazobactam *Enterobacteriaceae*	100/10 µg	≥21	18-20	≤17

Pseudomonas aeruginosa		≥21	15-20	≤14
Imipenem *Enterobacteriaceae*	10 µg	≥23	20-22	≤19
Pseudomonas aeruginosa		≥19	16-18	≤15
Meropenem *Enterobacteriaceae*	10 µg	≥23	20-22	≤19
Pseudomonas aeruginosa		≥19	16-18	≤15

*: intrinsic resistance for *Klebsiella pneumonia*

#: intrinsic resistance for *Pseudomonas aeruginosa*

Table 6: *Staphylococcus aureus*

Anibiotics	**Disc content**	**Resistant (mm)**	**Intermediate (mm)**	**Sensitive (mm)**
Penicillin	10 units	≥29	-	≤28
Amikacin	30 µg	≥17	15-16	≤14
Gentamicin	10 µg	≥15	13-14	≤12
Norfloxacin	10 µg	≥17	13-16	≤12

Nitrofurantoin	300 µg	≥17	13-16	≤14
Trimethoprim-sulfamethoxazole (Cotrimoxazole)	1.25/ 23.75 µg	≥16	11-15	≤10
Cefoxitin	30 µg	≥22	-	≤21
Tetracycline	30 µg	≥19	15-18	≤14

Table 7*: Enterococcus spp.*

Anibiotics	**Disc content**	**Resistant (mm)**	**Intermediate (mm)**	**Sensitive (mm)**
Penicillin	10 units	≥15	-	≤14
Tetracycline	30 µg	≥19	15-18	≤14
Norfloxacin	10 µg	≥17	13-16	≤12
Nitrofurantoin	300 µg	≥17	15-16	≤14
Vancomycin	30 µg	≥17	15-16	≤14
High level gentamicin	120 µg	≥10	7-9	≤6

2) Minimum inhibitory concentration (MIC)

a. MIC for Meropenem by macrobroth dilution method: [38]

MIC was determined for the isolates that showed resistance to meropenem by disc diffusion method

- ✓ **Culture media:** Cation adjusted Mueller Hinton broth (pH 7.2-7.4)

✓ **Antibiotic stock solution:** It was prepared using the formula:

$$W = \frac{1000 \times V \times C}{P}$$

where P= potency of the antibiotic in relation to the base. (For Meropenem, P= 675/1000 µg)

V = volume of the stock solution to be prepared (10ml)

C = final concentration of the antibiotic solution (1024µg/ml)

W = weight of the antibiotic to be dissolved in the volume V

15.17mg of drug was mixed with 10 ml of distilled water which contains 1024g/ml concentration of drug

✓ **Preparation of drug dilutions:**

A row of 15 sterile test tubes were arranged (one row for each isolate). One ml of MH broth was added to each tube upto 14th tube. From the stock solution (tube 1) 1ml was transferred to the second tube, then from the second to the third tube. This procedure was repeated till the thirteenth tube. One tube with only the antibiotic solution was kept as drug control.

✓ **Inoculum preparation for the test and ATCC control**

9.9 ml of MH broth was taken in a sterile test tube. 0.1ml of 0.5 Mc Farland turbidity matched test isolate suspension was added to it and mixed well. From this 1

ml of inoculum was transferred to each tube containing antibiotic dilutions. One tube containing only inoculum was kept as growth control also to the control tube.

Same procedure was repeated for ATCC control strain and incubated overnight at 37ºC.

✓ **Interpretation**- The lowest concentration of the antibiotic which showed clearing (no visible growth) was considered as the MIC for the ATCC strain & for the test organisms.

✓ **MIC of meropenem**:

≤2 µg/ml: susceptible

4 µg/ml: intermediate

≥8 µg/ml: resistant

b. <u>MIC for Vancomycin by macrobroth dilution method:</u> [37,38]

✓ **Procedure**- MIC for vancomycin was done in a similar way as for meropenem. However, the potency of vancomycin was taken as P= 950/1000 µg.

<u>Table 8: MIC range for Vancomycin</u>

Vancomycin	Resistant µg/ml	Intermediate µg/ml	Sensitive µg/ml
S.aureus	≥16	4-8	≤2
Enterococcus spp	≥32	8-16	≤4

c. <u>MIC for vancomycin using Epsilometer test:</u> [39]

In this method, MIC determination paper strip is used which is coated with vancomycin in a concentration gradient manner. It can show MIC's in the range of 0.016µg/ml to 256µg/ml on testing against the test organism.

✓ **Procedure-** A lawn culture of the test isolate was done on MHA. The E-strip was placed on the agar surface and the plate incubated at 37°C for 16-24 hours.

✓ **Interpretation-** MIC is read where the ellipse intersects the MIC scale on the strip. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition indicates heteronature of the culture having resistant sub-population in it. In such cases, MIC is read at a point on the scale above which no resistant colonies are observed close to MIC strip. The range for MIC is same as that for macrobroth dilution method.

3) <u>Detection of extended spectrum betalactamase (ESBL)</u>[37]

Screening of possible ESBL production was done by using cefotaxime (30µg) and ceftazidime (30µg) discs. Those isolates with zone diameters ≤ 27mm for ceftriaxone and ≤22 mm for ceftazidime were subsequently confirmed for ESBL production. Confirmation was done by ESBL phenotypic confirmatory test. A lawn culture of the test organism was done and ceftazidime (30µg) alone and ceftazidime plus clavulanic acid(30/10µg) discs were placed with centres 30mm apart. An increase of ≥ 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer.

4) <u>Detection of Amp C beta-lactamases</u> [40,41,60]

Screening for Amp C beta-lactamase was done using cefoxitin(30µg) disc. A zone diameter of ≤18mm was suspected as Amp C betalactamase producer. Such isolates were further confirmed by Amp C disc test.

A lawn culture of ATCC *E.coli* 25922 strain was streaked on an MHA plate. The inoculum was allowed to dry and a cefoxitin disc (30µg) was placed at the centre of the plate. A sterile disc almost touching to the cefoxitin disc was placed. It was moistened with 20 µl of sterile saline and then inoculated with 3-4 colonies of the test organism. The plate was incubated overnight at 37°C. A flattening or inhibition of the cefoxitin inhibition zone in the vicinity of the disc was considered a positive result

5) Detection of metallobetalactamase (MBL) [40,41,60]

Screening for MBL detection was done using imipenem (10µg) disc. Those isolates found resistant to imipenem were confirmed by imipenem EDTA combined disk test [(33)]. A lawn culture of the test isolate was done and the discs were placed with centres 30mm apart. A zone diameter difference of ≥ 7 mm between the imipenem and imipenem + EDTA (10/750µg) was interpreted as a positive result for MBL production.

6) Detection of carbapenemase resistance [37,41]

Those isolates which showed intermediate or resistant to one or more carbapenems (imipenem/meropenem) were tested for carbapenemase production by Modified Hodge test (MHT). A 0.5 McFarland suspension of E.coli ATCC strain was made in saline. It was diluted 1:10 in saline and then streaked on MH plate to produce a lawn culture. It was allowed to dry for 3-5 minutes and a meropenem disc (10 µg) was placed at the centre of the plate. Using a 10µl loop, 3-5 colonies of the test organism was picked up and inoculated in a straight line out from the edge of the disk. The streak

line should be at least 20-25mm in length. Control strain was also streaked. Up to 4 organisms can be tested on the same plate. The plate was incubated overnight for 16-24 hours at 37°C. A clover leaf-like indentation of the *E.coli* ATCC 25922 strain within the zone of inhibition of the carbapenem susceptibility was considered as positive MHT.

7) Detection of methicillin resistance *Staphylococcus aureus* (MRSA) [37]

A lawn culture of the test isolate was done and a cefoxitin disc (30µg) was placed. The plate was incubated overnight at 33-35°C. A zone of inhibition ≤21mm was taken as mec-A positive and considered as MRSA.

B) ANTIFUNGAL SUSCEPTIBILITY

1) Disc diffusion method[42]

Inoculum was prepared by suspending 4-5 colonies from 24 hour old culture grown on Sabouraud Dextrose Agar in 5 ml sterile saline (0.85%NaCl). The turbidity was adjusted to 0.5 McFarland standard.

Within fifteen minutes of suspension preparation, a sterile cotton-wool swab was dipped into it. The soaked swab was rotated against the upper inside wall of the tube to express excess fluid. Mueller Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue plate was inoculated by streaking the entire surface of the plate with the swab three times, turning the plate at 60° angle between each set of streaking.

The inoculum was allowed to dry for 5-15 minutes and then the discs were placed with centres at least 24mm apart. The control strain *C.albicans* ATCC 90028

was also put up. Antifungal discs used were Fluconazole (25µg) and Voriconazole (1 µg)

Table 9: Interpretative criteria for antifungal susceptibility

Antifungal drug	Disc potency	Zone diameter range		
		R	SDD	S
Fluconazole*	25µg	≤14	15-18	≥19
Voriconazole	1µg	≤13	14-16	≥17

*: Intrinsic resistance for *C.krusei*

2) Minimum inhibitory concentration (MIC) by microbroth dilution method [43]

- ✓ **Preparation of inoculum**

To obtain pure and viable cultures, the yeast isolates to be tested were subcultured on SDA. 4-5 colonies of 24 hour old culture was suspended in 5 ml sterile NaCl [0.85 %], vortexed for 15 seconds and the turbidity was adjusted to 0.5 McFarland standard. It was diluted 1:500 using RPMI-1640 medium i.e. 100 µl of yeast suspemsion is added to 5ml of RPMI1640 medium.

- ✓ **Weight of antifungal drugs**

$$\text{Weight (mg)} = \frac{\text{volume(ml) x concentration (µg/ml)}}{\text{Assay potency (µg/mg)}}$$

- ✓ **Preparation of stock solution**

All drugs are dissolved in 10 ml of DMSO to prepare the stock solution of the drug. Water soluble drug, fluconazole was dissolved in the concentration of 5120 µg/ml and water insoluble drugs like itraconazole and amphotericin B were dissolved in the concentration of 1600 µg/ml.

- ✓ **Preparation of drug dilutions**
  - ❖ **Intermediate drug concentration**

Water soluble drugs- Ten tubes were arranged in a row. **RPMI** was added in the following manner: 7ml to the first tube, 1ml to second, fourth, seventh and tenth tubes; 3 ml to the third tube; 1.5 ml to the fifth and eighth tubes and 3.5 ml to the sixth and ninth tubes.

The drug was added in the following manner: From the stock solution, 1ml of drug was added to the first tube. From the first tube 1 ml was transferred to second and third tubes. From the third tube, 1 ml was transferred to the fourth tube and 0.5 ml to the fifth and sixth tubes. From the sixth tube 1 ml was transferred to seventh tube and 0.5 ml to the eighth and ninth tube and from the ninth tube 1ml was transferred to tenth tube.

Water insoluble drugs- Ten tubes were arranged in a row. Stock solution 1600 µg was taken in first tube. DMSO was added in the following manner: 0.5 ml to the second, fifth and eighth tubes; 1.5 ml to the third, sixth and ninth tubes and 3.5 ml to fourth, seven and tenth tubes.

The drug was added in the following manner: 0.5 ml of the drug was transferred from the 1st tube to 2nd, 3rd and 4th tubes; 0.5 ml from the 4th tube to 5th, 6th and 7th tubes and 0.5 ml from 7th tube to 8th, 9th and 10th tubes.

- **Final drug concentration**

Water soluble drug- Another row of ten test tubes were arranged and 4 ml of RPMI was added to all the tubes. 1ml of intermediate drug concentration prepared were added to these tubes from the corresponding rows.

Water insoluble drug- Another row of ten test tubes are arranged. 4.9 ml of RPMI was added to all the tubes. 0.1ml of intermediate drug concentration prepared were added to these from the corresponding rows.

Table 10: Preparation of dilutions of water soluble antifungal drug

Tube	1	2	3	4	5	6	7	8	9	10
Source:	From stock	From tube 1	From tube 1	From tube 3	From tube 3	From tube 3	From tube 6	From tube 6	From tube 6	From tube 9
Add drug (ml)	1.0 +	1.0 +	1.0 +	1.0 +	0.5 +	0.5 +	1.0 +	0.5 +	0.5 +	1.0 +
Add solvent	7.0	1.0	3.0	1.0	1. 5	3. 5	1. 0	1.5	3.5	1.0

RPMI (ml)										
Intermediate drug concentration (row 1)	640	320	160	80	40	20	10	5	2.5	1.25
Add drug from tube in row 1 above (ml)	1.0 +	1.0 +	1.0 +	1.0 +	1.0 +	1.0 +	1.0 +	1.0 +	1.0 +	1.0 +
Add RPMI 1640 (ml)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Final concentration (µg/ml)	**64**	**32**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**0.125**

Table 11: Preparation of dilutions of water soluble antifungal drug

Tube	1	2	3	4	5	6	7	8	9	10
Source :	Stock	From tube 1	From tube 1	From tube 3	From tube 3	From tube 3	From tube 6	From tube 6	From tube 6	From tube 9
Add drug (ml)	-	0.5 +	0.5 +	0.5 +	0.5 +	0.5 +	0.5 +	0.5 +	0.5 +	0.5 +
Add solvent DMSO (ml)	-	0.5	1.5	3.5	0.5	1.5	3.5	0.5	1.5	3.5
Intermediate drug concentration	1600	800	400	200	100	50	25	12.5	6.25	3.13
Add drug from tube in row 1	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +

above (ml)										
Add RPMI 1640 (ml)	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
Final concentration (µg/ml)	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**0.125**	**0.0625**	**0.0313**

- ✓ **Inoculation of the plate and incubation**

A sterile flat bottomed 96 well microtitre plate was labelled. Wells 1 to 10 were labelled for drug concentrations: 64 to 0.125 for water soluble drug i.e. fluconazole and 16 to 0.0313 for water insoluble drugs i.e. itraconazole and amphotericin B; 11th well for growth control and 12th well for drug control. 100µl of each serial dilution of antifungal agents were added in the respective wells. 100µl of the inoculum was added to wells 1 to 10. To the 11th well, 100µl of RPMI and 100µl of the inoculum was added. To the 12th well, 100µl 0f RPMI and 100µl of drug from stock solution were added.

The microtitre plate was incubated at 35º C for 48 hours.

- ✓ **<u>Observation</u>**

Reading of results and scores:

1- Optically clear

2- Approximately 25% of the growth control or Slighty hazy

3- Approximately 50% of the growth control or prominent decrease in turbidity

4- Approximately as of the growth medium or slight reduction in turbidity

5- No reduction in turbidity

✓ **Interpretation**

The MIC for the drugs was the lowest concentration with a score of 3 for water soluble drugs and score of 1 for amphotericin B

Table-12: MIC range for Azole drugs by microbroth dilution method

Candida spp	**Resistant µg/ml**	**Susceptible dose dependent µg/ml**	**Sensitive µg/ml**
Fluconazole	≥64	16-32	≤8
Itraconazole	≥1	0.25-0.5	0.125
Amphotericin B	>1	-	≤1

RESULTS

STATISTICAL ANALYSIS:

Occurrence of symptomatic CAUTI was taken as primary outcome. Various personal (age, gender, etc) and clinical parameters (type of disease, steroid use, etc) of the patients were considered as explanatory factors. Descriptive analysis of all the explanatory and outcome parameters was presented as frequencies and percentages. The association between explanatory and outcome variables was analyzed by calculating odds ratios and their 95% confidence intervals. Statistical significance of this association was analyzed using chi square test. Microsoft excel and IBM SPSS version 21 were used for analysis.

RESULTS:

In this study, total of 100 patients were enrolled and included in the final analysis.

Table 13: Incidence of symptomatic CAUTI

Total Number of cases with Symptomatic CAUTI	26 (26%)
Total Duration of catheterisation (days)	1013
Symptomatic CAUTI rate per 1000 catheter days	26/1013*1000=25.67

The incidence rate of CAUTI per 1000 catheter days was calculated as 25.67 cases in the study population.

Table 14: Descriptive analysis of age-wise distribution in study group (N=100)

Age Groups	Frequency	Percent
18 to 30 yrs	33	33.0
31 to 40 yrs	24	24.0
41 to 50 yrs	18	18.0
51 to 60 yrs	11	11.0
61 to 70 yrs	10	10.0
71 to 80 yrs	4	4.0

The age distribution of the participants showed, maximum proportion (33%) of subjects belonging to 18 to 30 years. The proportion of subjects with age between 31 to 40 years, 41 to 50 yrs, 51 to 60 years, 61 to 70 yrs and 71 to 80 yrs was 24%, 18%, 11% 10 and 4% respectively.

Fig 4: Pie chart of age groups distribution in study group (N=100)

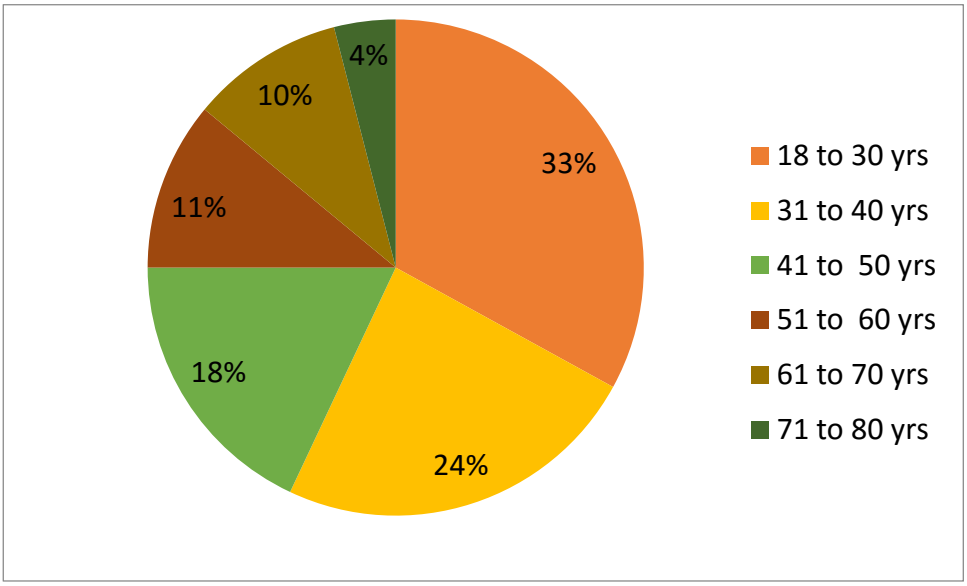


Table 15: Descriptive analysis of gender distribution in study group (N=100)

Gender	Frequency	Percent
Male	57	57.0
Female	43	43.0

Males constituted 57% and females contributed 43% of study subjects.

Fig 5: Bar chart of gender distribution in study group (N=100)

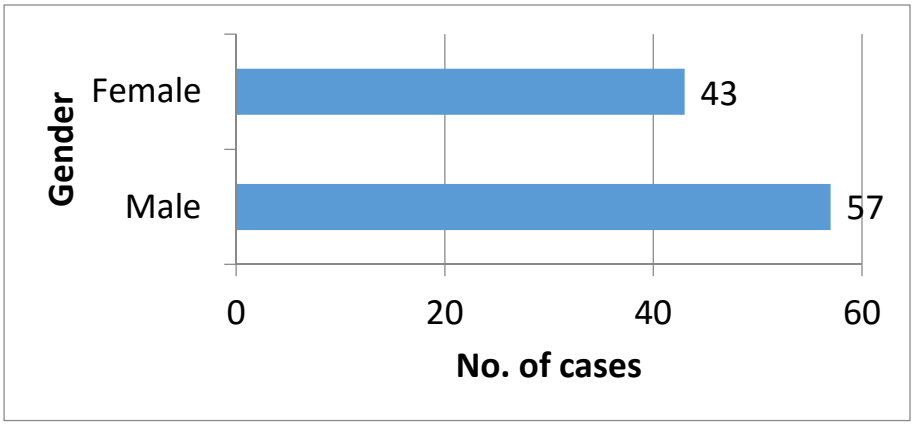


Table 16: Descriptive analysis of Total catheter days in study group (N=100)

Total catheter days	Frequency	Percent
1 to 7 Days	11	11.0
8 to 14 days	85	85.0
15 to 21 days	4	4.0
Total	100	100.0

In majority of Patients total catheter days were in the range of 8 to 14 days (85%).In 1 to 7 days range 11 (11%) and in 15 to 21 days only 4(4%) patients were there.

Fig 6: Pie chart of total catheter days distribution in study group (N=100)

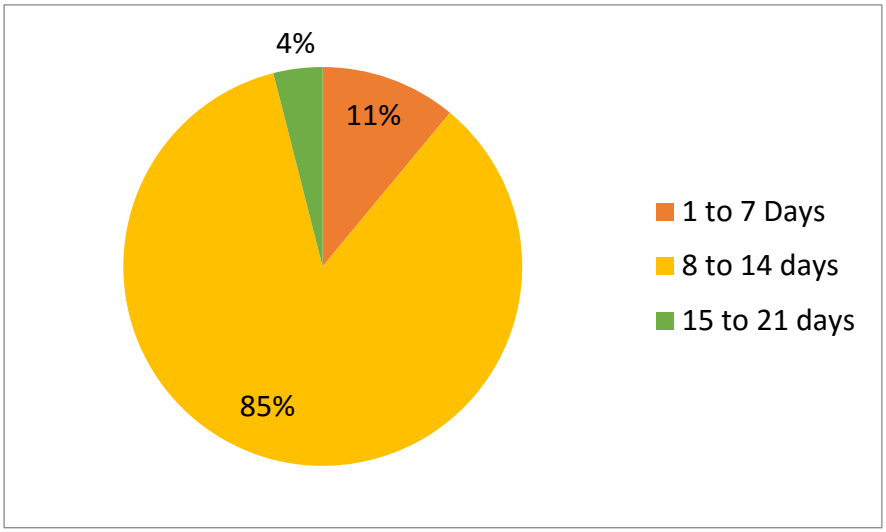


Table 17: Descriptive analysis of Indication for catheterisation and validity in study group (N=100)

Parameter	Frequency	Percent
I. Indication for catheterisation		
Monitor urinary output	96	96.0
Monitor urinary output, relieve urinary retention	4	4.0
II. Indication valid		
Yes	100	100.0
No	0	0.0

The indication for catheterisation was valid for all cases

Fig 7: Bar chart of indication for catheterisation distribution in study group (N=100)

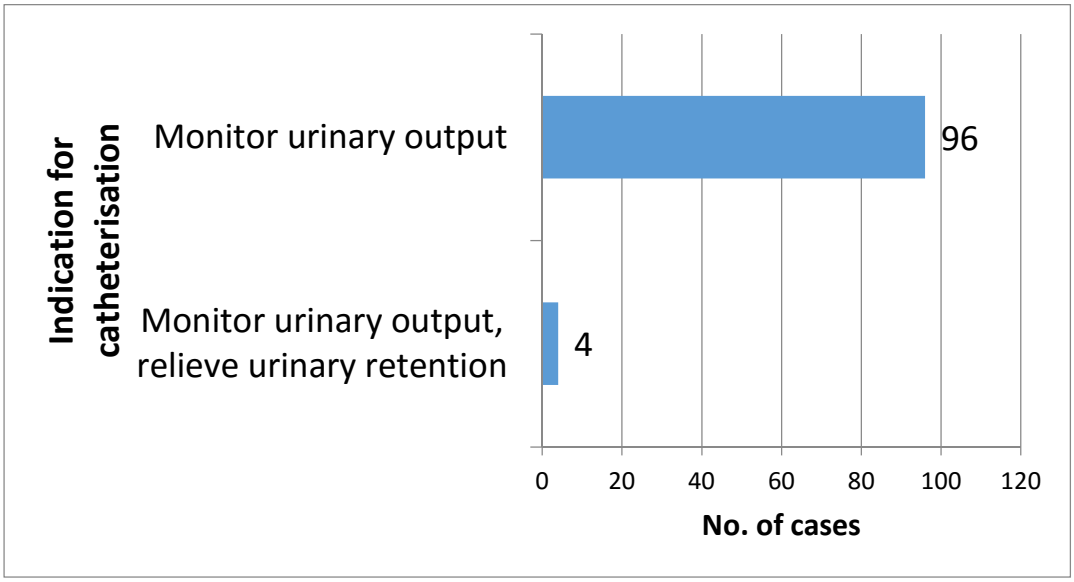


Table 18: Descriptive analysis of CAUTI parameters in study group (N=100)

Parameter	Frequency	Percent
I. Symptomatic CAUTI		
Yes	26	26.0
No	74	74.0
II. Day of development of Symptomatic CAUTI (N=26)		
10	4	15.3
14	19	73.0
21	3	11.5

A total of 26 (26%) of subjects developed symptomatic CAUTI during the hospital stay. Out of symptomatic CAUTI, majority 19 (73%) developed it on 14th day, followed by

4(15.3%), who developed on day 10. Remaining 3(11.5%) subjects developed CAUTI on 21st day.

Fig 8: Bar chart of Symptomatic CAUTI distribution in study group (N=100)

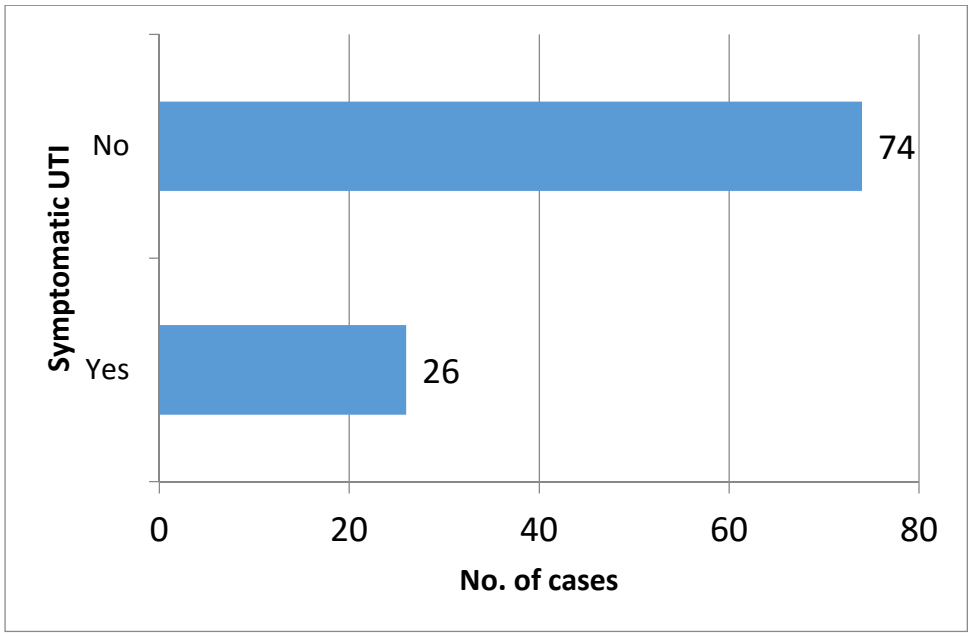


Fig 9: Pie chart of day of development of Symptomatic CAUTI distribution in study group (N=26)

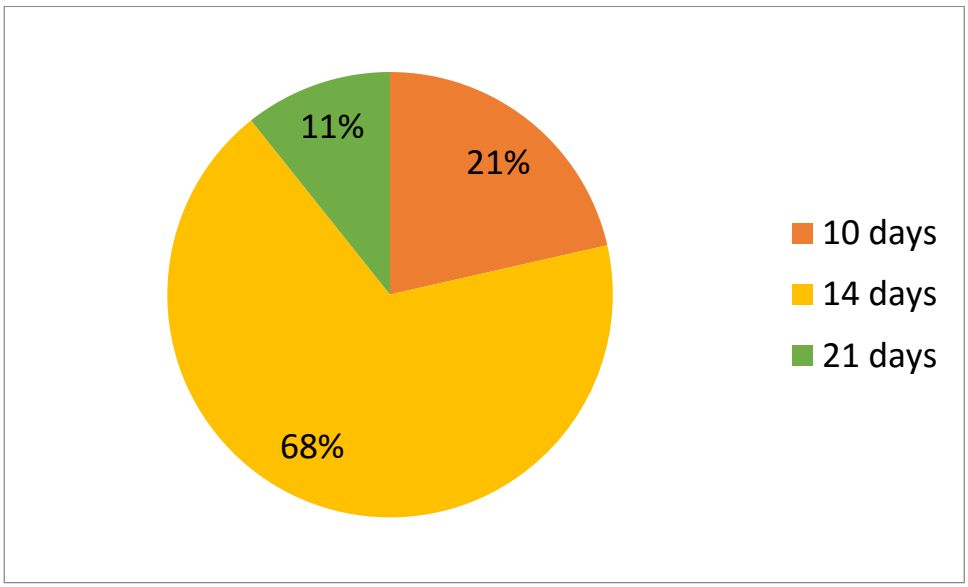


Table 19: Descriptive analysis of day of development of symptomatic CAUTI in study group (N=100)

Parameter	Frequency	Percent
I.@3 days		
Yes	0	0.0
No	100	100.0
II. @5 days		
Yes	0	0.0
No	100	100.0
III. @ 7 days		
Yes	0	0.0
No	100	100.0
IV.@ 10 days (N=63)		
Yes	4	6.3
No	59	93.6
V. @ 14 days (N=22)		
Yes	19	86.3
No	3	13.6
VII. @21 days (N=4)		
Yes	3	75.0
No	1	25.0

Table 20: Descriptive analysis of Risk factors in study group (N=100)

Parameter	Frequency	Percent
I. Age 50yrs and above		
Yes	26	26.0
No	74	74.0
III. Duration of catheterisation ≥10days		
Yes	43	43.0
No	57	57.0
IV. **Diabetes mellitus**		
Yes	17	17.0
No	83	83.0
V. **Neurological causes**		
Yes	18	18.0
No	82	82.0
VI. Respiratory causes		
Yes	11	11.0
No	89	89.0
VII. Urological Nephrological causes		
Yes	5	5.0
No	95	95.0
VIII. Steroid		
Yes	5	5.0

No	95	95.0
IX. Other immunocompromised conditions		
Yes	5	5.0
No	95	95.0
X. Faulty catheter care		
Yes	3	3.0
No	97	97.0

The descriptive analysis of all the potential risk factors for development of symptomatic CAUTI is presented in table 7. A total of 26(26%) of participants were aged above 50 years. The proportion of subjects who had catheterization for more than 10 days was 43% .17 % of subjects had Diabetes mellitus. The proportion of subjects, who were suffering from neurological, respiratory conditions, was 18% and 11% respectively. Only 5% of subjects were suffering from urological/nephrological conditions and 5 patients each had steroid use and other immunocompromised conditions. 3 patients each had faulty catheter care.

Fig 10:Bar chart of Risk factors distribution in study group

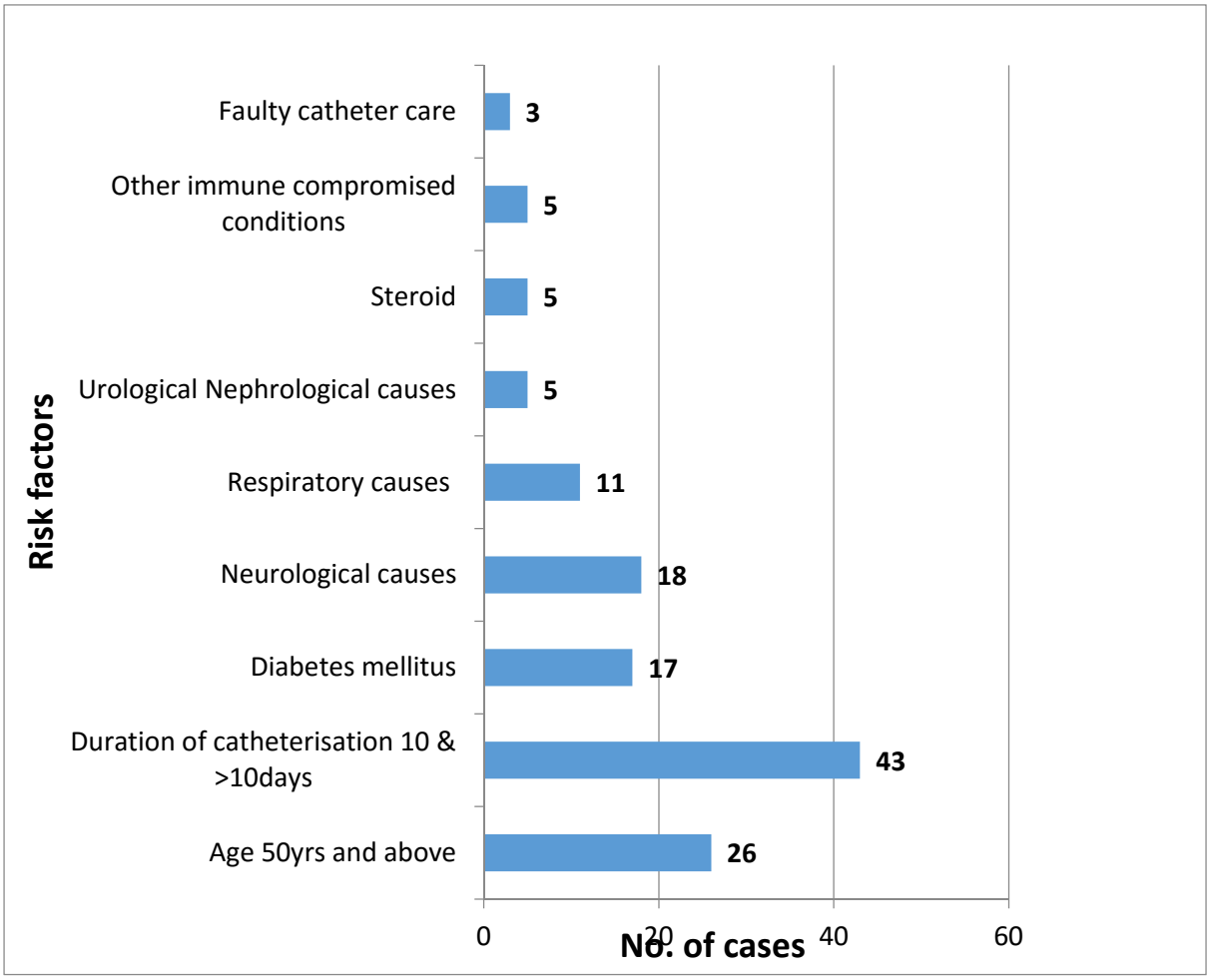


Table 21: Association between Age groups and CAUTI in study group (N=100)

Parameter	Symptomatic CAUTI		Chi square value	P value
	Yes	No		
Age groups				
18 to 30 yrs	5 15.15%	28 84.85%		
31 to 40 yrs	5	19	10.722	0.057

	20.83%	79.17%			
41 to 50 yrs	8 44.44%	10 55.56%			
51 to 60 yrs	3 27.3%	8 72.7%			
61 to 70 yrs	2 20.0%	8 80.0%			
71 to 80 yrs	3 75.0%	1 25.0%			

The occurrence of CAUTI was found highest in age group 71-80 years. However, the association was not statistically significant between the age groups and occurrence of CAUTI in the study population.

Table 22: Patient personal and clinical factors influencing in study group (N=100)

Parameter	**Symptomatic CAUTI**		**Odds ratio**	**Chi square value**	**P value**	**95% CI**	
	Yes	No				**Lower**	**Upper**
1.Age 50yrs and above							
Yes	8	18	1.383	0.415	0.519	0.515	3.713
	30.77%	69.23%					
No	18	56					
	24.32%	75.68%					

II. Gender							
Female	15	28	2.240	3.094	0.079	0.903	5.559
	34.88%	65.12%					
Male	11	46					
	19.30%	80.70%					
III.Duration of catheterisation ≥10days							
Yes	26	17	-	46.574	0.000	0.273	0.572
	60.47%	39.53%					
No	0	57					
	0.00%	100.00%					
VIII. Steroid use							
Yes	2	3	1.972	0.536	0.464	0.311	12.519
	40.00%	60.00%					
No	24	71					
	25.26%	74.74%					
X. Faulty catheter care							
Yes	1	2	1.440	0.086	0.769	0.125	16.574
	33.3%	66.7%					
No	25	72					
	25.8%	74.2%					

The odds of occurrence of symptomatic CAUTI were 1.38 times in people above 50 years, compared with people below 50 years, which was statistically not significant. (P

value 0.5, 95% CI 0.51 to 3.71). Female gender had 2.2 times higher risk of developing CAUTI, compared to males (p value 0.07, 95 CI 0.90 to 5.55). None of the patients catheterized for less than 10 days had CAUTI. The patients who were on steroids had 1.97 times more risk of developing UTI, compared to other people (P value0.464, 95% CI 0.311 to 12.519). The patients with faulty catheter care had 1.440 times risk of developing CAUTI. However it was not statistically significant (P value 0.769, 95% CI 0.125. to 16.574)

Table 23: Association between morbidity and symptomatic CAUTI in study group (N=100)

Parameter	**Symptomatic CAUTI**		**Odds ratio**	**Chi square value**	**P value**	**95% CI**	
	Yes	No				**Lower**	**Upper**
IV. Diabetes Mellitus							
Yes	10	7	5.982	11.469	0.001	1.973	18.138
	58.82%	41.18%					
No	16	67					
	19.28%	80.72%					
V. Neurological causes							
Yes	10	8	5.156	9.966	0.002	1.754	15.157
	55.56%	44.44%					
No	16	66					
	19.51%	80.49%					

VI. Respiratory causes							
Yes	7	4	6.447	9.099	0.003	1.707	24.353
	63.6%	36.4%					
No	19	70					
	21.35%	78.65%					
VII. Urological Nephrological causes							
Yes	4 80.00%	1 20.00%	13.273	7.977	0.005	1.409	124.994
No	22 23.16%	73 76.84%					
IX. Other immunocompromised conditions							
Yes	2	3	1.972	0.536	0.464	0.311	12.519
	40.00%	60.00%					
No	24	71					
	25.26%	74.74%					

The odds of symptomatic CAUTI were 5.98 times more in diabetic people (P value <0.001, 95 CI 1.97 to 18.13). People suffering from neurological and respiratory diseases had 5.16 (p value 0.002, 95 CI 1.75 to 15.15) times and 6.44 (P value 0.003, 95% CI 1.70 to 24.35) times more risk of symptomatic CAUTI. People with urological/nephrological condition had the highest risk of suffering from symptomatic CAUTI, with an odds ratio of 13.27 (P value 0.003, 95% CI 1.4 to 124.99). The persons

with other immunocompromised conditions had no statistically significant increased risk of CAUTI.

Table 24: Descriptive analysis of Organism isolated in study group (N=40)

Organism Isolated	Frequency	Percent
Candida Species	6	15.0
Gram negative bacilli		
Enterobacteriaceae	14	34.5
Non-fermenters	13	32.5
Gram positive organisms	7	17.5
Total	40	100.0

Majority of the organisms isolated belonged to Enterobacteriaceae (34.5%) and non-fermenters (32.5%). Candida species (15.0%) and Gram positive organisms (17.5%) contributed to the remaining portion of the organisms.

Fig 11: Pie chart organism isolated distribution in study group (N=100)

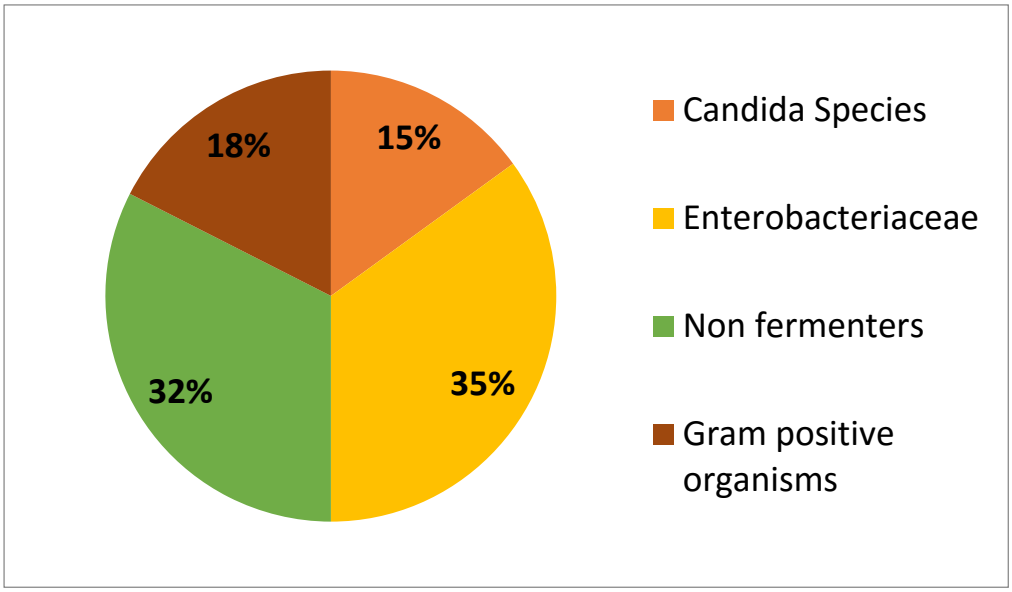
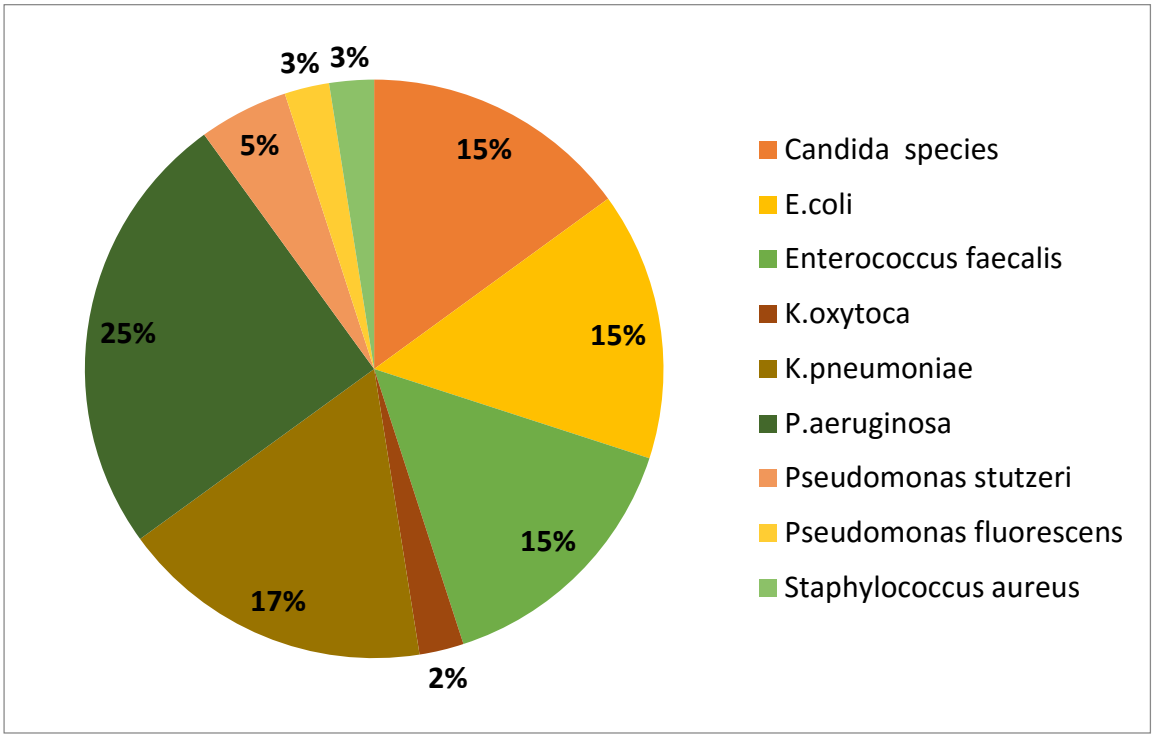


Table 25: Descriptive analysis of Organism isolated in study group (N=40)

Organism Isolated	Frequency	Percent
Candida species	**6**	**15**
Enterobacteriaceae		
Escherichia coli	6	15.0
Klebsiella oxytoca	1	2.5
Klebsiella pneumoniae	7	17.5
Non-fermenters		
Pseudomonas aeruginosa	10	25.0
Pseudomonas stutzeri	2	5.0
Pseudomonas fluorescens	1	2.5
Gram Positive organisms		

Staphylococcus aureus	1	2.5
Enterococcus faecalis	6	15.0
Total	40	100.0

Fig 12: Pie chart for distribution of organisms isolated in study group (N=100)

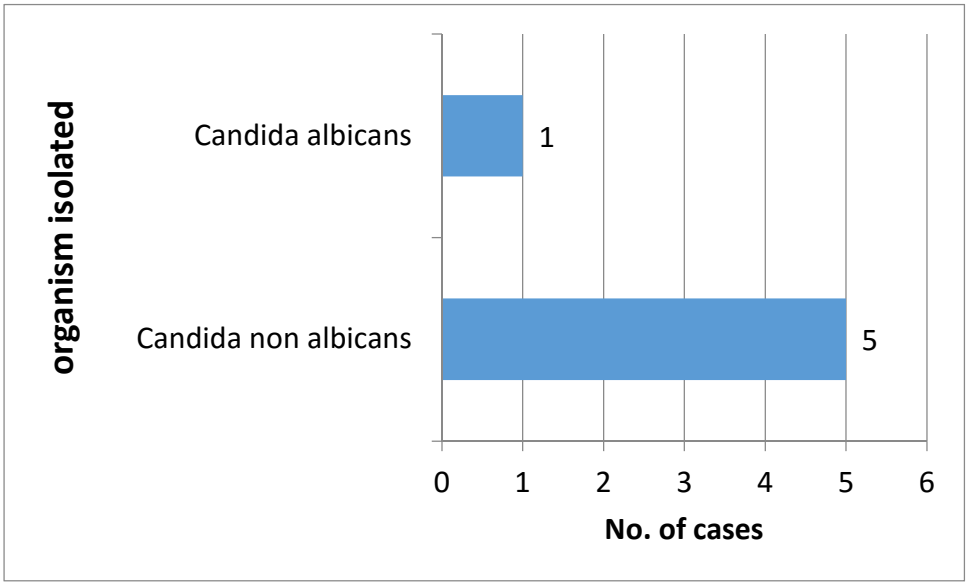


Pseudomonas aeruginosa was the most common isolate (25%) followed by *Klebsiella pneumoniae* (17.5%), *Enterococcus faecalis* (15%), *Escherichia coli* (15%) and Candida spp. (15%). Other isolates were *Pseudomonas stutzeri* (5%), *Klebsiella oxytoca* (2.5%), *Pseudomonas fluorescence* (2.5%). and *Staphylococcus aureus* (2.5%).

Table 26: Descriptive analysis of Candida spp. isolated in study group (N=6)

Organism Isolated	Frequency	Percent
Candida albicans	1	16.7
Non-albicans Candida	5	83.3
Total	6	100.0

Fig 13: Bar chart organism isolated distribution in study group (N=100)



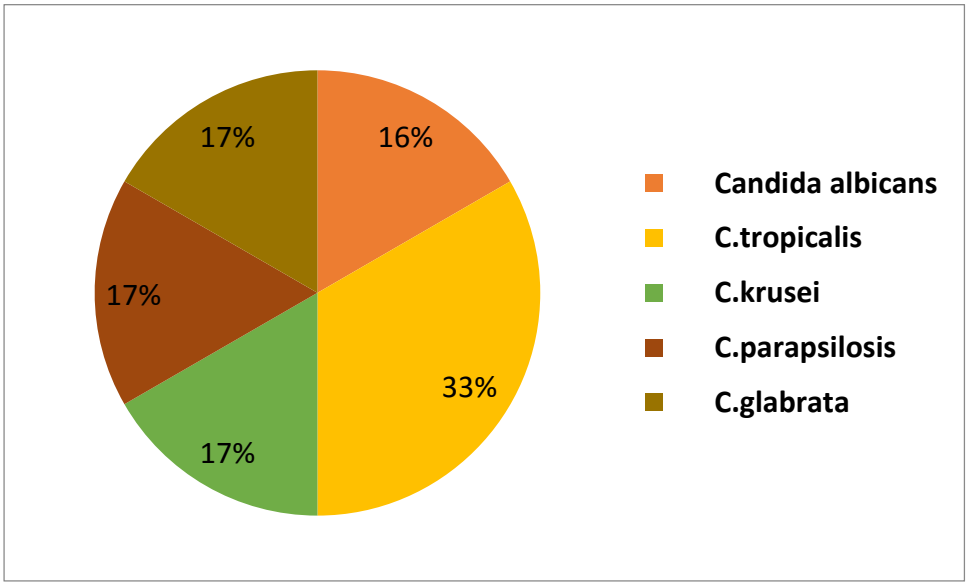
Among the candida species, non-albicans Candida spp. contributed to 83.3% of the isolates and only 16.7% of isolates were *Candida albicans*

Table 27: Descriptive analysis of various species of Candida isolated in study group (N=6)

Candida species	Frequency	Percentage
Candida albicans	1	16.67

C.tropicalis	2	33.33
C.krusei	1	16.67
C.parapsilosis	1	16.67
C.glabrata	1	16.67

Fig 14: Pie chart of Candida species isolated distribution in study group (N=6)



Among non-albicans Candida, 2 patients had Candida tropicalis and one patient each had Candida krusei, Candida parapsilosis and Candida glabrata isolate.

Table 28:Descriptive analysis of antimicrobial sensitivity pattern for Enterobacteriaceae in study group (N=14)

Drug	***Escherichia coli*** **(n=6)**	***Klebsiella oxytoca*** **(n=1)**	***Klebsiella pneumoniae*** **(n=7)**

Ampicillin	0 (0%)	0 (0%)	*
Amikacin	0 (0%)	0 (0%)	0 (0%)
Gentamicin	1 (16.7%)	0 (0%)	0 (0%)
Nitrofurantoin	3 (50%)	1 (100%)	1 (14.3%)
Norfloxacin	0 (0%)	0 (0%)	1 (14.3%)
Cotrimoxazole	0 (0%)	0 (0%)	0 (0%)
Cefotaxime	0 (0%)	0 (0%)	0 (0%)
Ceftazidime	0 (0%)	0 (0%)	0 (0%)
Piperacilin-tazobactam	5 (83.3%)	1 (100%)	3 (42.9%)
Imipenem	6 (100.0%)	1 (100%)	5 (71.4%)
Meropenem	6 (100%)	1 (100%)	5 (71.4%)
Tetracycline	0 (0%)	0 (0%)	1 (14.3%)

*: intrinsic resistance

There was a high degree of resistance observed among Enterobacteriaceae. However, they were commonly sensitive to piperacillin-tazobactam, imipenem and meropenem.

Table 29: Descriptive analysis of antimicrobial sensitivity pattern for Non-fermenters in study group (N=13)

Drug	**Pseudomonas aeruginosa (n=10) N(%)**	**Pseudomonas stutzeri (n=2) N(%)**	**Pseudomonas fluorescens (n=1) N(%)**
Amikacin	1 (10%)	0 (0%)	0 (0%)

Gentamicin	1 (10%)	0 (0%)	0 (0%)
Norfloxacin	2 (20%)	0 (0%)	0 (0%)
Cotrimoxazole	*	0 (0%)	0 (0%)
Cefotaxime	*	0 (0%)	0 (0%)
Ceftazidime	1 (10%)	0 (0%)	0 (0%)
Piperacilin-tazobactam	7 (70%)	1 (50%)	1 (100%)
Imipenem	9 (90%)	2 (100%)	1 (100%)
Meropenem	9 (90%)	2 (100%)	1 (100%)
Tetracycline	*	0 (0%)	0 (0%)

*: intrinsic resistance

Among Pseudomonas aeruginosa isolates, majority of them were sensitive to Imipenem (90%), meropenem (90%) and Piperacilin-tazobactam (70%). Among *Pseudomonas stutzeri* and *P.fluorescens* isolates, all the isolates were sensitive to imipenem and meropenem. Only 50% of *Pseudomonas stutzeri* were sensitive to Piperacilin-tazobactam.

Table 30:Descriptive analysis of antimicrobial sensitivity pattern for Enterococcus species in study group (N=6)

Drug	Sensitive N (%)
Nitrofurantoin	4 (66.7%)
Norfloxacin	3 (50.0%)
Tetracycline	1 (16.7%)
Penicillin	0 (0.0%)
Vancomycin	6 (100.0%)
High level gentamicin	5 (83.3%)

Among 6 isolates of Enterococcus species 100% were sensitive for vancomycin. The proportion of isolates sensitive for nitrofurantoin, norfloxacin, tetracycline and high level gentamicin was 66.7%, 50% , 16.7% and 83.3% respectively. None of the isolates were sensitive to penicillin.

Table 31: Descriptive analysis of drug sensitivity for Staphylococcus aureus in study group (N=1)

Drug	Sensitive, N (%)
Amikacin	0 (0.0%)
Gentamicin	0 (0.0%)
Nitrofurantoin	1 (100.0%)

Norfloxacin	0 (0.0%)
Cotrimoxazole	0 (0.0%)
Cefoxitin	0 (0.0%)
Tetracycline	1 (100.0%)
Vancomycin	1 (100.0%)
Penicillin	0 (0.0%)
Linezolid	1(100.0%)

Staphylococcus aureus isolate was sensitive to nitrofurantoin, tetracycline, vancomycin and linezolid.

Table 32: Descriptive analysis of drug sensitivity for *Candida spp.* in study group (N=6)

Drug	**Resistance N (%)**	**Sensitive N (%)**
Fluconazole*	0(0.0%)	5 (100.0%)
Voriconazole	0 (0.0%)	6 (100.0%)
Amphotericin B	0 (0.0%)	6 (100.0%)
Itraconazole	0 (0.0%)	6 (100.0%)

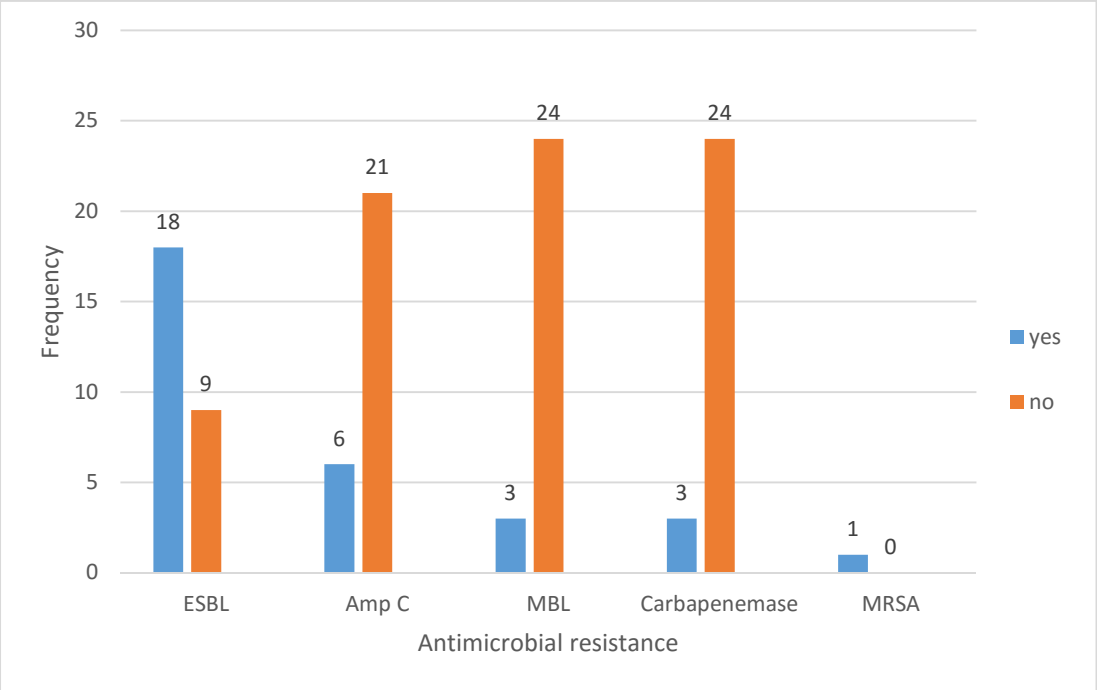
*: intrinsic resistance for *C.krusei*

All the candida isolates were sensitive to fluconazole, voriconazole, amphotericin B and itraconazole.

Table 33: Descriptive analysis of antibiotic resistant pattern in organisms isolated

Parameter	Frequency	Percent
I. ESBL(n=27)		
Yes	18	**66.67**
No	9	33.33
Total	27	100
II. Amp C (n=27)		
Yes	6	**22.22**
No	21	77.78
Total	27	100
III. MBL (n=27)		
Yes	3	**11.11**
No	24	88.89
Total	27	100
IV. Carbapenemase (n=27)		
Yes	3	**11.11**
No	24	88.89
Total	27	100
V. MRSA(n=1)		
Yes	1	**100**
No	0	0

Fig 15: Antimicrobial resistance pattern of the organisms isolated

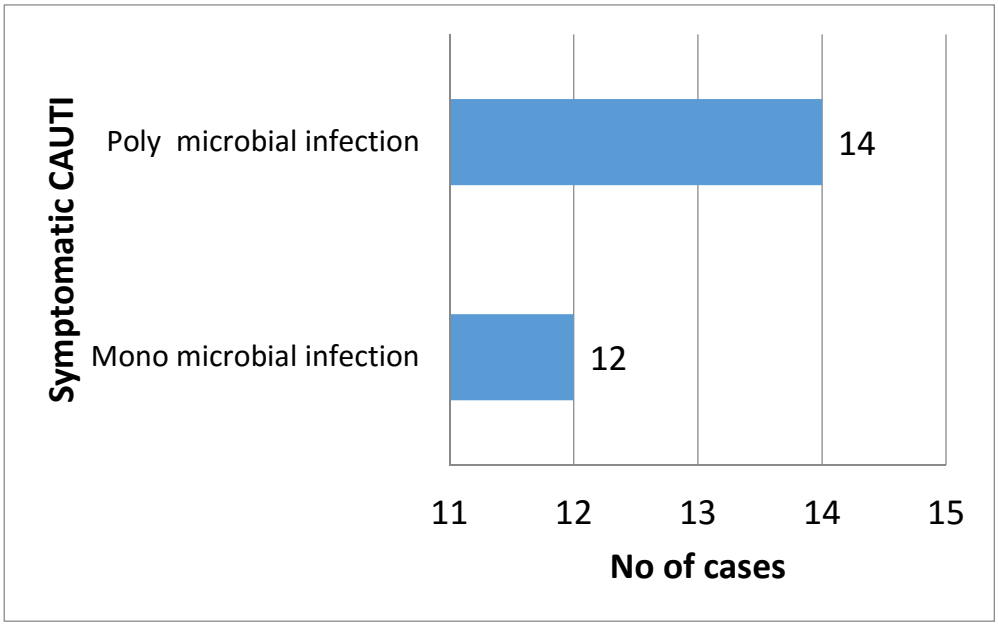


Among 27 isolates of Gram negative bacilli, 66.67 % for ESBL, 22.22% Amp C, 11.11 % MBL and 11.11% were positive for carbapenemase production. One S*taphylococcus aureus* was isolated which was methicillin resistant.

Table 34: Descriptive analysis of Type of Symptomatic CAUTI in study group (N=26)

Type of Symptomatic CAUTI	Frequency	Percent
Mono microbial infection	12	46.15
Poly microbial infection	14	53.84
Total	26	100.0

Fig 16: Bar chart of Symptomatic CAUTI distribution in study group (N=26)



Among the 26 cases of CAUTI, 12 (46.15%) were monomicrobial infections and remaining 14 (53.84%) were poly microbial infections.

Table 35: Distribution of organisms in polymicrobial infections

S.no.	Organism 1	Organism 2	Frequency
1	*Escherichia coli*	*Enterococcus faecalis*	1
2	*Klebsiella pneumoniae*	*Enterococcus faecalis*	1
3	*Pseudomonas aeruginosa*	*Candida krusei*	1
4	*Candida albicans*	*Enterococcus faecalis*	1
5	*Klebsiella pneumonia*	*Pseudomonas fluorescens*	1
6	*Escherichia coli*	*Candida parapsilosis*	1
7	*Pseudomonas aeruginosa*	*Candida tropicalis*	2
8	*Klebsiella pneumonia*	*Pseudomonas stutzeri*	1
9	*Escherichia coli*	*Klebsiella pneumonia*	1

10	*Pseudomonas aeruginosa*	*Candida glabrata*	1
11	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	1
12	*Pseudomonas aeruginosa*	*Escherichia coli*	2

Table 36: Distribution of organisms in monomicrobial infections

S.no.	Organism	Frequency
1.	*Escherichia coli*	1
2.	*Klebsiella pneumoniae*	2
3.	*Klebsiella oxytoca*	1
4.	*Pseudomonas aeruginosa*	3
5.	*Pseudomonas stutzeri*	1
6.	*Staphylococcus aureus*	1
7.	*Enterococcus faecalis*	3

DISCUSSION

Catheter associated urinary tract infection is the commonest device associated nosocomial infection. A total of 100 patients admitted in medical ICU were enrolled in the study and followed up for the development of CAUTI. In this study, only symptomatic cases were included under CAUTI.

The rate of device associated infections shows variation in India. According to a study conducted by Angshuman Jana et al (2015) [44], the incidence was 31.85%. Another study by Neha Garg et al (2015) [45] found the incidence to be 20%. A study by Priya Datta et al (2014) [46] found the CAUTI rate as 10.75% and 9.08/1000 catheter days by Pooja et al (2014) [47] as 32.14%, by Kamat et al (2009) [48] as 33.6%, C.M.Poudel et al (2008) [8] as 54% and Al Jebouri et al (2006) [49] as 28.1 %. Habibi et al (2008) [50] conducted a study in AIIMS, Delhi and reported CAUTI as 24% of nosocomial UTIs, the rate being 11.3/1000 catheter days. Study conducted in ICUs of four Mexican public hospitals reported CAUTI as 21.79% [51]**.** A survey conducted to determine the DAIs in the ICUs of 8 different developing countries reported that CAUTI comprised 29% of all DAIs (52). Due to these wide variations in the incidence, it is important for a hospital to generate its own data for the implementation on proper infection control programmes.

In this study, out of 100 patients, 26 patients were diagnosed to develop symptomatic CAUTI during their course of hospitalisation. Therefore, the incidence was 26% and the CAUTI rate was calculated as 25.67 per 1000 catheter days.

The age distribution of the study subjects showed maximum proportion (33%) of the patients belonged to 18-30 years. Males constituted 57% and females 43% of the

study subjects. In majority of the patients (85%), total catheter days were in the range of 8-14 days. The indication of catheterisation was found to be valid in all patients. Among 26 patients who developed symptomatic CAUTI, 19 developed in day 14.

A number of risk factors implicated with the development of symptomatic CAUTI were studied .The p value and Odd's ratio were calculated by Chi square test to find the statistical significance ($p<0.05$) and the strength of association of these risk factors. Age ≥ 50 years showed increased development of CAUTI, the risk being 1.38 times. The incidence was also higher among females (34.88%) than males (19.3%). However, in this study age and gender showed no statistical significance. Similar results were seen in studies conducted by Priya et al [46], Meric et al [53] and Agrawal et al [54].

Duration of catheterisation and length of hospital stay constitute an important risk factor and has been cited in studies by Priya Datta et al [46] and Angshuman Jana et al [44]. In this study, maximum patients (85%) belonged to the category of duration of catheterisation for 8 to 14 days. Among patients catheterised for 14 days, 86.3% developed CAUTI and among 4 patients catheterised till 21 days, 75% developed the infection. Duration of catheterisation 10 ≥days was found to be statistically significant as among 43 patients who had catheter for 10 ≥days, 60.47% developed CAUTI. This is due to the fact that the longer a patient stays in the ICU and catheterised, more are the chances that he will get colonised with multidrug resistant organisms present in the environmental niche.

Faulty catheter care is another risk factor, but in this study it was not found to be statistically significant.

Co-morbidities have significant association with the development of CAUTI. In this study, diabetes had 5.98 times the risk, neurological causes 5.16 times, respiratory conditions such as COPD 6.44 times and those with urological/ nephrological causes had 13.27 times the risk. All these co-morbidities were statistically significant. These results are similar to study by Priya Datta et al [46] where diabetes and COPD had significant association.

Infection by two organisms is common in CAUTI. In the study polymicrobial infection was seen in 53.84% and monomicrobial in 46.15 % of the cases. Total of 40 organisms were isolated.

In this study, the predominant isolates were Gram negative bacilli comprising 67% of the isolates among which Enterobacteriaceae were 34.5% and non-fermenters 32.5%. This finding was similar to other studies where in GNB constituted the common isolate: Neha Garg et al (80%) [45], Priya Datta et al (72.61%) [46] and C.M.Poudel et al (66.67%) [8].

The organisms causing CAUTI vary from one geographical area to another and there is changing trend over a period of time. A prospective study conducted by Tullu MS et al (1998) [55] found the commonest organism was *Escherichia coli*. Wazait et al (2003) [16] although reported similar result, noticed a declining trend over the period time. *Enterococcus spp.* was isolated as the second commonest organism. Another study by Taiwo et al (2006) [55] revealed multiresistant *Klebsiella spp.* as the commonest isolate followed by *Pseudomonas spp., Escherichia coli, Staphylococcus aureus*, Proteus mirabilis and coagulase negative staphylococci. A study by Priya Datta et al (2014) [46] found *Pseudomonas aeruginosa* (35.7%) as the commonest isolate followed

by *Enterococcus spp*.(15.4%) and *Klebsiella pneumoniae* (15.4%), *Candida spp*.(11.9%), *Escherichia coli* (10.7%), *Acinetobacter spp* (9.5%) and *Morganella morganii* (1.1%). Study by Neha Garg et al (2015) [45] found *Escherichia coli* as the commonest isolate(40%) followed by *Citrobacter koseri* (20%), *Staphylococcus aureus* (15%), *Klebsiella oxytoca* (10%), *Acinetobacter spp* (5%). *Pseudomonas aeruginosa* (5%) and *Enterococcus faecalis* (5%). According to a study by Angshuman Jana et al (2015) [44], the main pathogen belonged to Enterobacteriaceae among which *Escherichia coli* was 19.4%. Other organisms included *Pseudomonas spp.* 19.4% followed by *Klebsiella spp*. (16.6%), *Staphylococcus* and *Candida spp*.(11%) each.

In the present study, *Pseudomonas aeruginosa* was the commonest isolate (25%) followed by *Klebsiella pneumonia* (17.5%), *Escherichia coli, Enterococcus faecalis* and *Candida spp.* (15%) each. *P.stutzeri* comprised 5% and *Klebsiella oxytoca*, *P.fluorescens* and *Staphylococcus aureus* 2.5% each. Among the gram positive bacteria, *Enterococcus faecalis* comprised 6 isolates and *Staphylococcus aureus* one isolate. There is an increasing trend of Enterococcus faecalis causing CAUTI.

Among *Candida* isolates, *non-albicans Candida* spp. (83.3%) emerged as the predominant isolate. These included *Candida tropicalis* (2), *C.parapsilosis* (1), *C.krusei* (1) and *C.galbrata* (1). These findings were similar to the findings in a study conducted by Manisha Jain et al (2011) [57] where *non-albicans Candida spp.* (71.4%) was the predominant pathogen causing CAUTI. Similar results were seen in a study by Chanda R. Vyawahare et al (2015) [58]. *Non-albicans Candida* spp. are thus replacing *Candida albicans* as the predominant pathogen for nosocomial UTI.

Different studies have shown high degree of antibiotic resistance among the pathogens causing CAUTI such as studies by Angshuman et al (2015) [44], Neha Garg et al(2015) [45], Priya Datta et al(2014) [46] and Chanda R. Vyawahare et al (2015) [58]. It is reported that catheterisation increases the prevalence of UTI caused by some highly resistant pathogens and the resistant pattern of the isolates changes from time to time.[16]. In the present study, among Enterobacteriaceae, the organisms were mostly resistant to ampicillin, amikacin, gentamicin, nitrofurantoin, norfloxacin, cotrimoxazole, tetracycline, cefotaxime and ceftazidime. For piperacillin-tazobactam, sensitivity was more – *E.coli*(83.3%), *K.oxytoca* (100%) and *K.pneumoniae* (42.9%). All isolates were uniformly sensitive to imipenem and meropenem except one isolate of *K.pneumoniae* which was resistant to carbapenems. Among non-fermenters, *P.aeruginosa* was predominantly sensitive to piperacillin-tazobactam, imipenem and meropenem. Among gram positive cocci, *E.faecalis* was 100% sensitive to vancomycin, 83.3% to high level gentamicin, 66.7% to nitrofurantoin and 50% to norfloxacin. Low level of sensitivity was seen for tetracycline (16.67%). One isolate of *S.aureus* was MRSA which was sensitive to nitrofurantoin, tetracycline, vancomycin and linezolid.

All the *Candida spp.* were uniformly sensitive to antifungals- fluconazole, itraconazole, voriconazole and amphotericin B. This may be due to the fact that the routine use for antifungal is not much. So, the organisms have not developed resistance.

Antimicrobial resistance is the main concern in healthcare associated infections because of the rapid increasing incidence. In this study, 66.67% of the GNB isolates were ESBL producers, 22.22% Amp C producers and 11.11% were positive for metallobetalactamase and carbapenemase each. This finding was similar to the study by

Mita et al (2013) [59]. In another study conducted by Neha Garg et al (2015) [45] , ESBL production was found in 25% of the strains, 37.5% of the isolates were positive for Amp C production and MBL was detected in 18.7% of the isolates.

In the study, one Staphylococcus aureus isolated was MRSA and there was no vancomycin resistant enterococci.

SUMMARY

- A total of 100 patients admitted in IMCU and put on Foley's catheter were included in the study.
- These patients were followed up for the development of symptomatic CAUTI. The urine samples from the catheter were collected on day 1 and then on day 3,5,7,10,14 and every weekly till the patient was discharged, expired, catheter removed or developed bacteriuria.
- Total 26 patients developed Symptomatic CAUTI, thereby the incidence being 26 % and the CAUTI rate was calculated as 25.67 per 1000 catheter days.
- The age distribution of the study subjects showed maximum proportion (33%) of the patients belonged to 18-30 years.
- Males constituted 57% and females 43% of the study subjects. In majority of the patients (85%), total catheter days were in the range of 8-14 days.
- The indication of catheterisation was found to be valid in all patients.
- Among 26 patients who developed symptomatic CAUTI, 19 developed in day 14.
- Various risk factors associated with symptomatic CAUTI were studied. Statistically significant risk factors included duration of catheterisation ≥10 days and comorbid conditions such as diabetes mellitus, neurological causes, respiratory causes and urological/nephrological causes.
- Polymicrobial infection was seen in 53.84% and monomicrobial in 46.15 % of the cases. Total of 40 organisms were isolated.

- In this study, the predominant isolates were Gram negative bacilli comprising 67% of the isolates among which *enterobacteriaceae* were 34.5% and non-fermenters 32.5%
- *Pseudomonas aeruginosa* was the commonest isolate (25%) followed by *Klebsiella pneumoniae* (17.5%), *Escherichia coli, Enterococcus faecalis* and *Candida spp*. (15%) each. *P.stutzeri* comprised 5% and *Klebsiella oxytoca*, *P.fluorescens* and *Staphylococcus aureus* 2.5% each.
- High degree of antibiotic resistance was observed among the pathogens causing symptomatic CAUTI. On the whole the bacterial isolates were more sensitive to piperacillin-tazobactam, imipenem and meropenem.
- ESBL production was found in 25% of the strains, 37.5% of the isolates were positive for Amp C production and MBL was detected in 18.7% of the isolates.
- One *Staphylococcus aureus* isolated was MRSA
- There was no vancomycin resistant *enterococci*.
- Among *Candida* isolates, *non-albicans Candida spp.* (83.3%) emerged as the predominant isolate
- No resistance was observed among candida isolates

CONCLUSION

This cross-sectional study conducted at Madras Medical College and Rajiv Gandhi Government General Hospital aimed at detecting one of the most common healthcare associated infection i.e. symptomatic catheter associated urinary tract infection. Development of CAUTI is common in critically ill patients. Emphasis should be placed on good catheter management and reducing the duration of catheterization rather than prophylaxis in order to reduce the incidence of catheter-related UTI. Culture and susceptibility testing play an important role in the management of CAUTI.

The assessment of risk and need of catheterisation should be evaluated. The indwelling catheter should be used in the patients only if there is a valid indication. It should be removed when it is no longer indicated. If the catheter is required for more than 14 days, it should be replaced or alternative methods of catheterisation such as condom catheter, etc. should be considered. In catheterised patients, proper catheter bundle care should be followed.

Antimicrobial resistance is a growing threat worldwide. There is an increasing resistance to third generation cephalosporins among Gram negative bacilli. The prevalence of extended spectrum betalactamases, Amp C betalactamases and metallobetalactamases constitutes a serious threat to current -lactam therapy leading to treatment failure. There is increase in the emergence of multidrug resistant isolates causing CAUTI. In order to decrease the incidence of drug resistance, prophylactic use of antibiotics should be discouraged. Knowledge of resistant pattern can help in implementing proper antibiotic therapy and infection control policy such as avoidance of overuse of antimicrobials, use of drugs for which pathogens are sensitive.

Fig 1:Foley's Catheter and urinary bag

Fig 2:Direct Gram stain: Gram negative bacilli

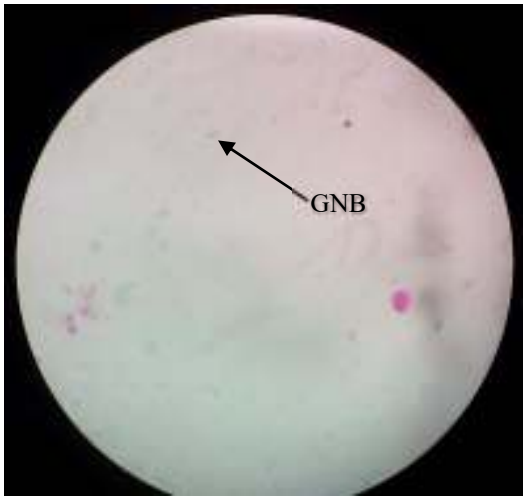
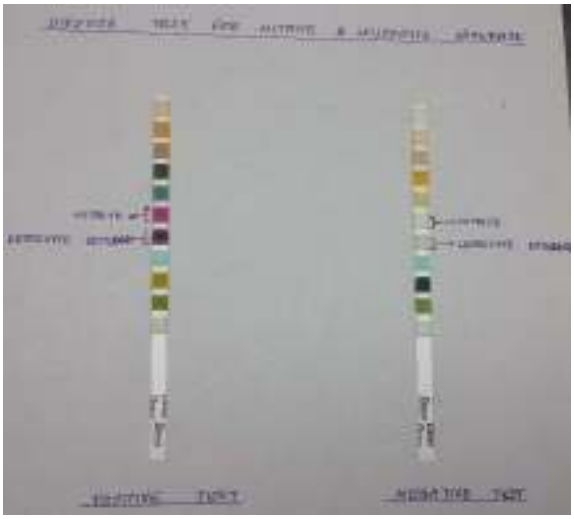


Fig 3:Direct Gram stain: Gram positive budding yeast cell

Fig 4: Wet mount: Pus cells

Fig 5:Dipstick test



Streaking by calibrated loop method

Fig 6:Mac Conkey Agar- Lactose fermenting colonies Blood agar- Grey white colonies

Fig 7:MAC- Non-lactose fermenting colonies BAP- Grey white colonies

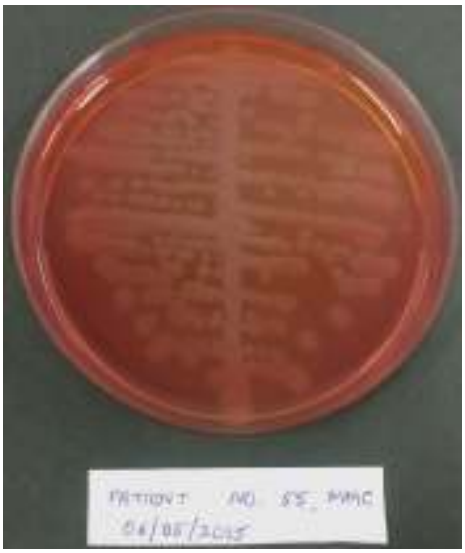
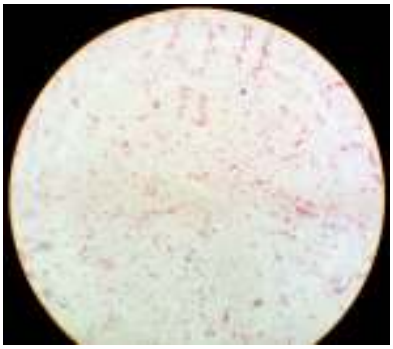
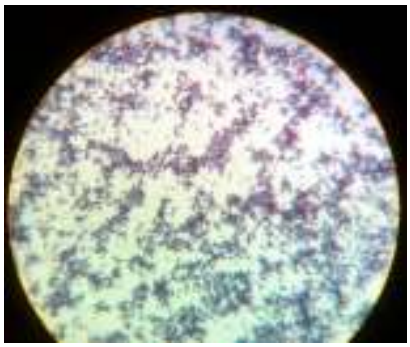


Fig 8:Culture Smears





Gram negative Bacilli **Gram positive cocci in clusters** Gram positive budding yeast cells with pseudohyphae

Fig 9:Catalase test

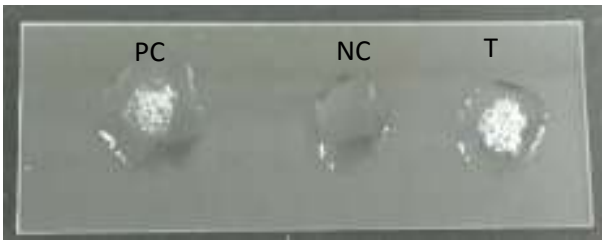


Fig 10:Oxidase test

Fig 11:Biochemical reactions of Pseudomonas aeruginosa

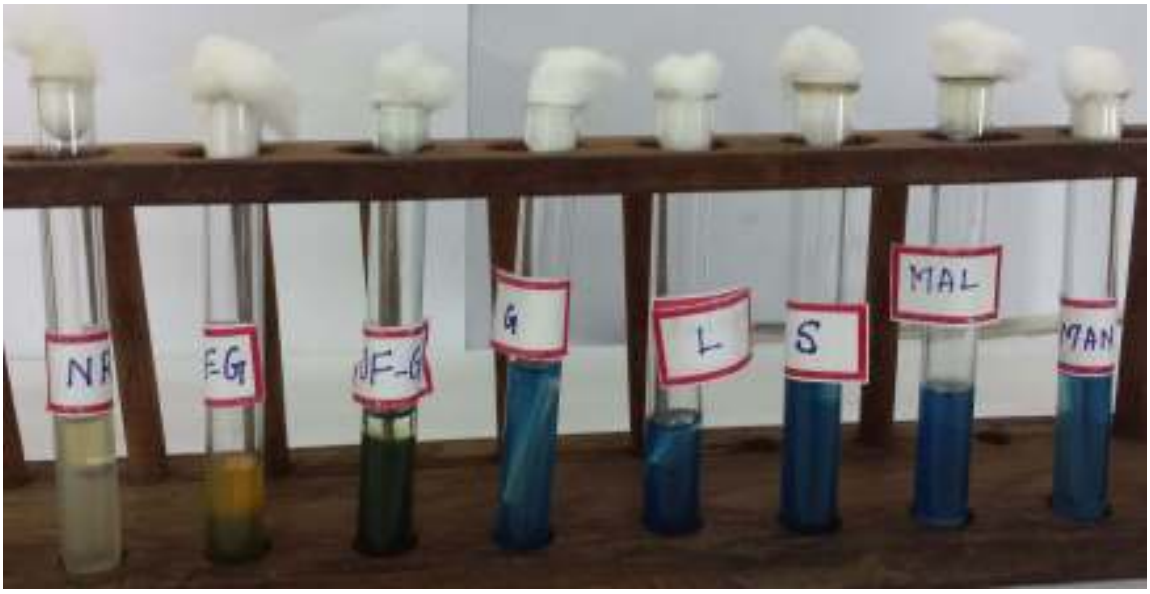


Fig 12:Biochemical reactions of *Escherichia coli*

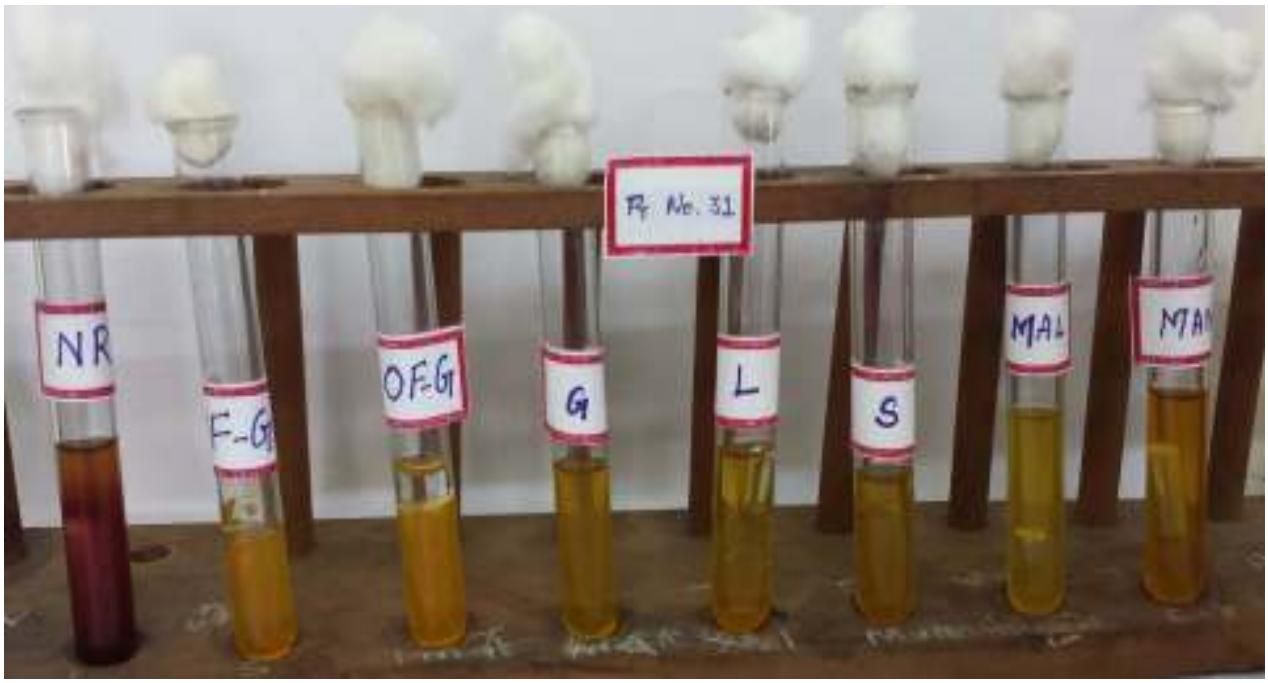


Fig 13:Biochemical reactions of *Klebsiella pneumoniae*

Fig 14:Biochemical reactions of *Enterococcus faecalis*

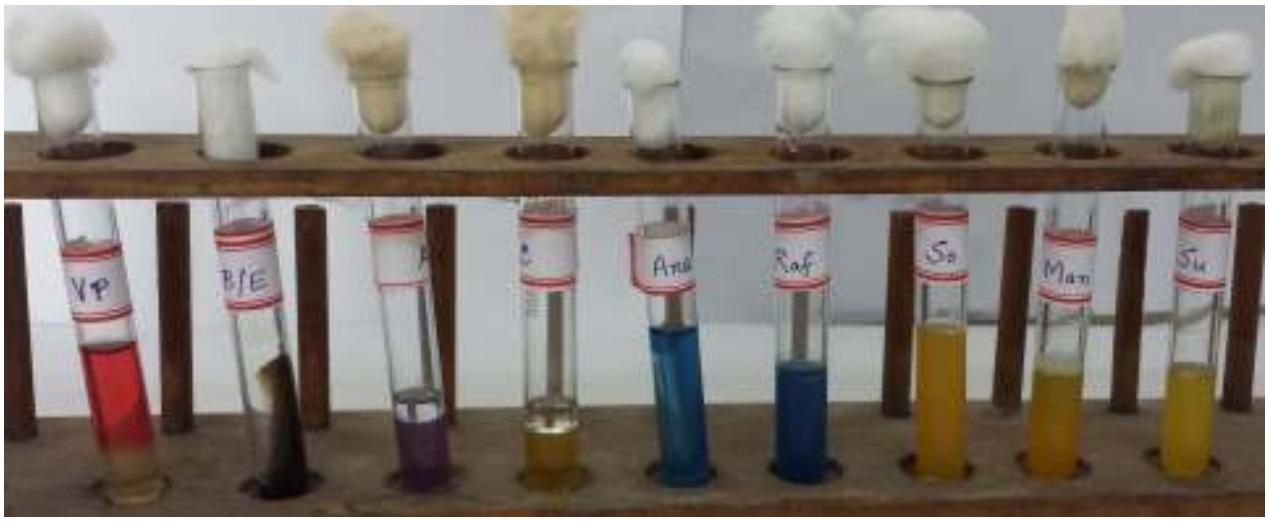


Fig 15:Antimicrobial susceptibility for Gram negative bacilli

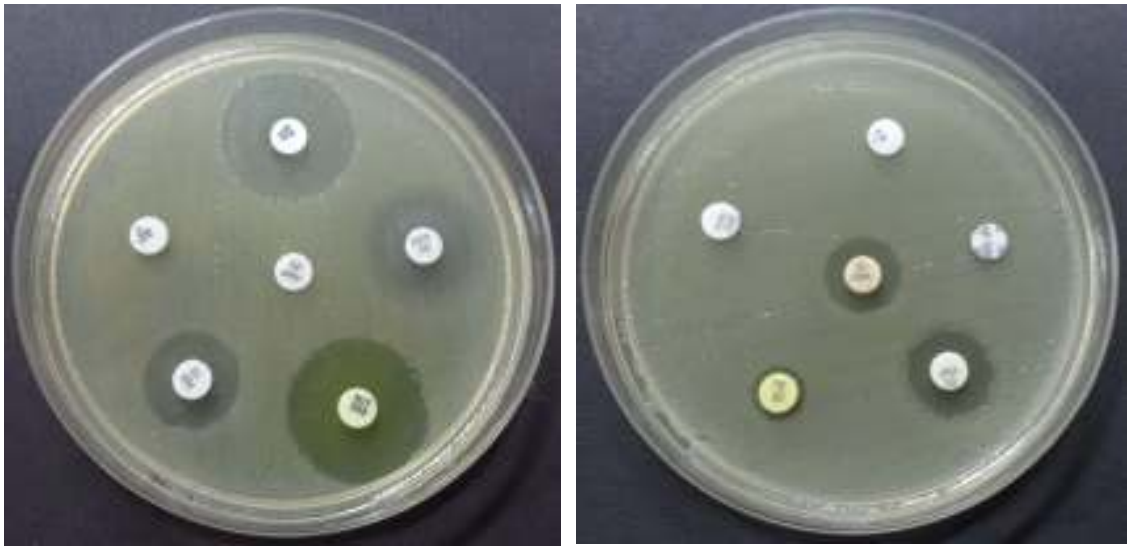


Fig 16:Antimicrobial susceptibility for Enterococcus spp.

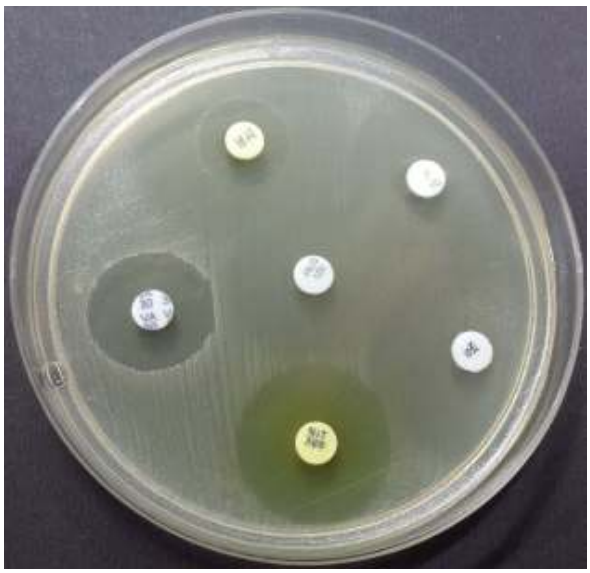


Fig 17:Antibiogram for *S.aureus*

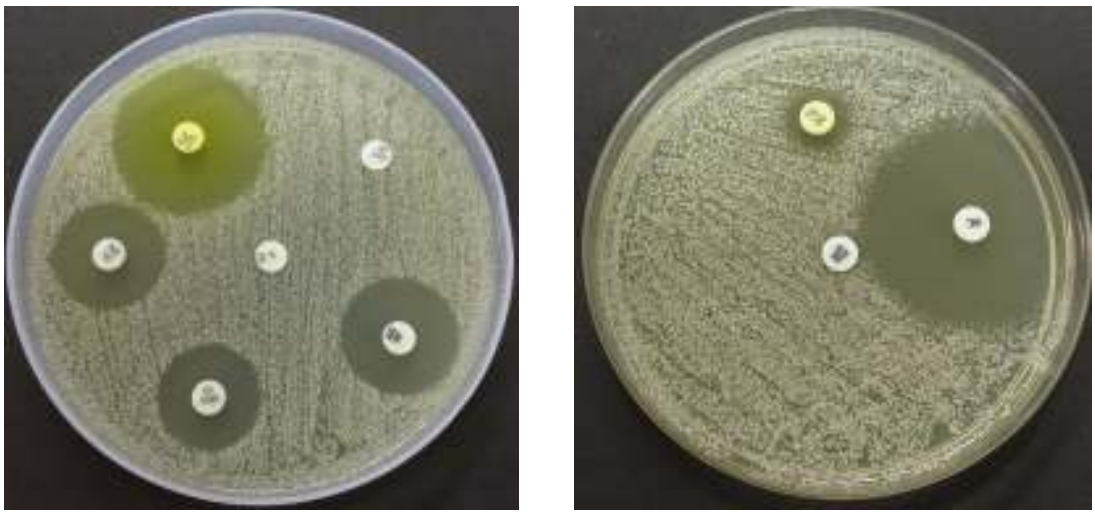


Fig 18:Minimum inhibitory concentration for Meropenem by macrobroth dilution method

Fig 19: Minimum inhibitory concentration for Vancomycin by Macrobroth dilution method

Fig 20: MIC for vancomycin by Epsilometer test

Fig 21: ESBL detection

Fig 22: Amp C detection

Fig 23:Metallobetalactamase detection

Fig 24: Germ tube test for Candida:

Positive **Negative**

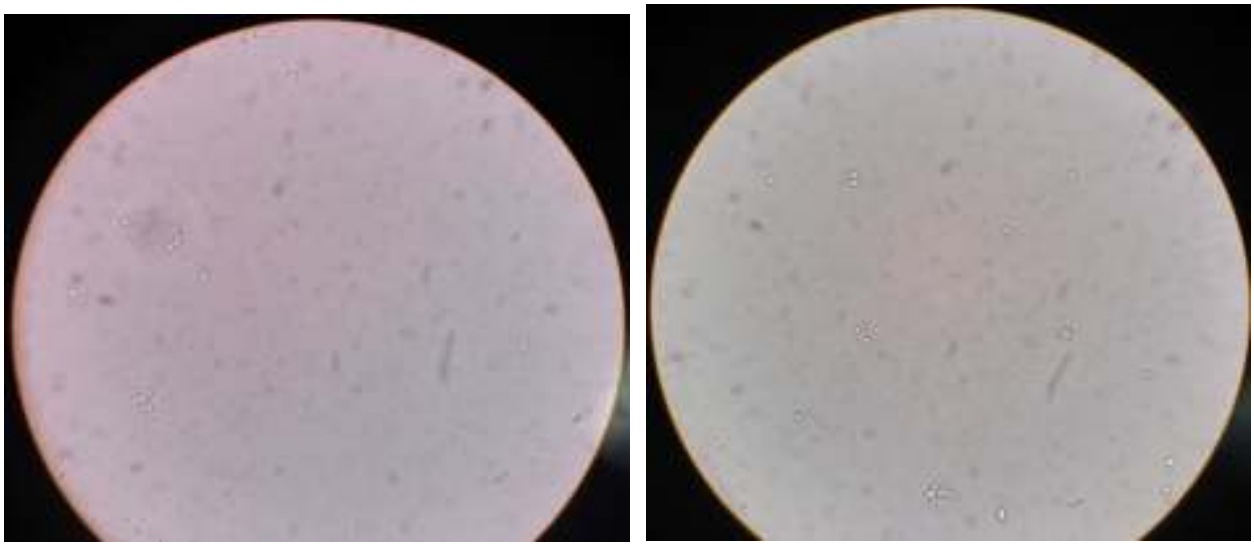


Fig 25: Candida Chrom Agar **Fig 26: Sugar fermentation test**

Fig 27: Sugar assimilation test

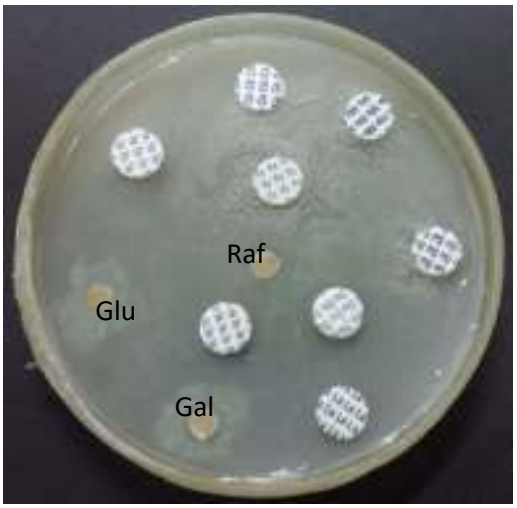


Fig 28:Cornmeal Tween 80 agar: *C.tropicalis* *C.krusei*

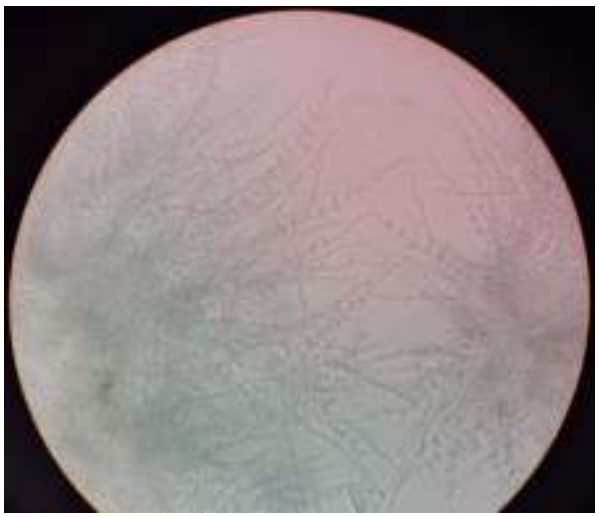


Fig 29:Antifungal susceptibility test

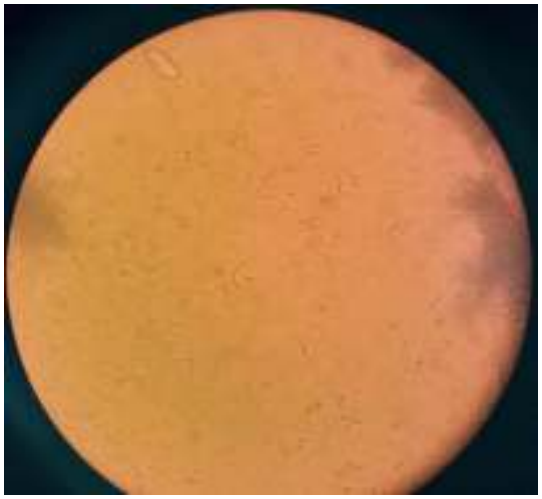
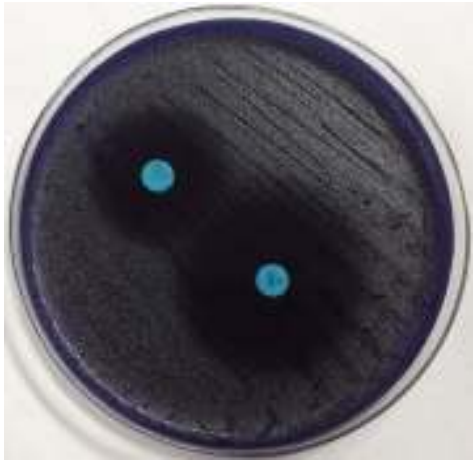


Fig 30:MIC for antifungal drugs



APPENDIX – I

ABBREVIATIONS

ATCC	American Type Culture Collections
BAP	Blood agar plate
ESBL	Extended spectrum Beta Lactamases
CAUTI	Catheter associated urinary tract infection
Cel	Cellobiose
CFU	Colony Forming Units
CLED	Cysteine lactose electrolyte deficient
CLSI	Clinical & Laboratory Standards Institute
COPD	Chronic obstructive pulmonary disease
DMSO	Dimethyl Sulfoxide
Dul	Dulcitol
Gal	Galactose
GNB	Gram negative bacilli
Glu	Glucose
HAI	Healthcare associated infection
hpf	High power field
ICU	Intensive care unit
IMCU	Intensive medical are unit
Ino	Inositol
Lac	Lactose
Mal	Maltose
MIC	Minimum Inhibitory Concentration

MBL	Metallobetalactamases
OIF	Oil immersion field
PMN	Polymorhonuclear neutrophils
Raff	Raffinose
RPMI	Rose Well Park Memorial Institute
SDA	Sabouraud's Dextrose Agar
Suc	Sucrose
Tre	Trehalose
UTI	Urinary Tract Infection
WBC	White blood cell
Xyl	Xylose

APPENDIX II

A. STAINS AND REAGENTS

1. Gram staining

Methyl violet (2%) - l0g Methyl violet in 100ml absolute alcohol in 1 litre of distilled water (primary stain).

Grams Iodine - l0g Iodine in 20g KI (fixative)

Acetone - Decolourising agent

Carbol fuchsin 1% - Secondary stain.

B. MEDIA USED

1. Mac Conkey Agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1 ltr
Agar	20g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	100 ml

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix. Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

2. 5% Sheep Blood Agar

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	l0g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

3. Sabourauds Dextrose agar

Dextrose	20 gm
Peptone	10 gm
Agar	20 gm
Distilled Water	1000 ml

Final pH : 6.9

The ingredients were dissolved by boiling. Chloramphenicol(50mg/lit) and Cycloheximide(500mg/lit) was added . Chloramphenicol was dissolved in 10 ml of 95% ethanol and added to boiling medium. Cycloheximide was dissolved in 10 ml of acetone and added to the boiling medium. It was autoclaved at 121°C for 15 minutes and dispensed in sterile tubes and allow to cool in slanted position.

4. Mueller Hinton Agar

Beef Infusion	300ml
Caesein Hydrolysate	17.5g
Starch	1.5g

Agar	20 g
Distilled Water	1ltr

ph = 7.4

Sterilise By autoclaving at 121°C for 20 minutes.

5. Corn meal tween 80 agar medium

Cornmeal : 8gm

Agar : 4gm

Tween 80(1%): 2ml

Distilled water: 200ml

Heat cornmeal and water at 60ºC for 1 hour and filter through filter paper.Add distilled water to make it 200ml and then add agar.Tween 80 is then added.Autoclave it at 121 ºC for 15 mins.

6. Yeast nitrogen base medium

Part A : Agar 40gms/lit

Part B : Yeast nitrogen base 6.7gms/lit

40 grams of part A media is suspended in 900 ml of distilled water. Heat to boiling to dissolve the medium completely. Autoclave at 121º C for 12 minutes. Cool to 50° C and mixed with sterile part B solution aseptically.

7. Candida chromagar medium

Ingredients	Gms/L
Peptone	15.00
Yeast extract	4.00
Dipotassium hydrogen phosphate	1.00

Chromogenic mixture	7.22
Chloramphenicol	0.50
Agar	15.00

Final pH: 6.3

42.72 grams of media is suspended in 1000 ml of distilled water. Heat to boiling to dissolve the medium completely. Do not autoclave. Cool to 50° C and pour in sterile petridish.

8. Supplemented mueller hinton agar

Ingredients	Gms / Litre
Beef extract	3.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

Final pH (at 25°C) 7.3±0.2

38grams of media is suspended in 1000 ml of distilled water. Add 20gm of glucose(2%) and methylene blue (0.5µg/ml) is added. Dissolve the medium completely. Dispense and sterilize by autoclaving at 115-121°C for 10 minutes. Do not overheat.

9. RPMI 1640 MEDIUM [With glutamine and without bicarbonate]

Obtained commercially as a dehydrated powder. Suspend 8.4gms of media in 900ml of sterile distilled water. Stir it to completely dissolve the medium do not heat. Sterilize the medium by filtration. Final pH=7.0.

C. REAGENTS & MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1. Oxidase Reagent

Tetra Methyl P-Phenylene Diamine Dihyrochloride- 1% Aqueous Solution

2.Catalase test

3% Hydrogen Peroxide

3.Kovac's Reagent

Amyl Or Isoamyl Alcohol	150ml
Para Dimethyl Amino Benzaldehyde	10g
Concentrated Hydrochloric Acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4.Christensen's Urease Test Medium

Peptone	1g
Sodium Chloride	5g
Dipotassium Hydrogen Phosphate	2g
Phenol Red	6ml
Agar	20g
Distilled Water	1 Ltr
10% Sterile Solution Of Glucose	10ml
Sterile 20% Urea Solution	100ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to ph 6.8-6.9 and sterilize by autoclaving in a flask at 121°c for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5.Simmon's Citrate Medium

Koser's Medium	1 Ltr
Agar	20 g
Bromothymol Blue 0.2%	40ml

Dispense, autoclave at 121°c for 15 min and allow to set as slopes.

6.Triple Sugar Iron Medium

Beef Extract	3g
Yeast Extract	3g
Peptone	20g
Glucose	1g
Lactose	10 g
Sucrose	10g
Ferric Citrate	0.3g
Sodium Chloride	5g
S Odum Thiosulphate	0.3g
Agar	12g
Phenol Red 0.2% Solution	12ml
Distilled Water	1 Ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°c for 15 min and cool to form slopes with deep butts.

7. Glucose Phosphate Broth

Peptone	5g
Dipotassium Hydrogen Phosphate	5g
Water	1 ltr
Glucose 10% Solution	50ml

Dissolve the peptone and phosphate and adjust the ph to 7.6. Filter dispense in 5ml amounts and sterilize at 121°c for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

8.Peptone Water Fermentation Test Medium.

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with durham's tube. Basal medium peptone water sugar solutions:

Sugar	1ml
Dislilled Water	100ml

Ph = 7.6.

9.Potassium Nitrate Broth

Potassium Nitrate (KNO_3)	0.2gm
Peptone	5.0gm
DistilledWater	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

10.Phenyl Alanine Deaminase Test

Yeast Extract	3g
Dl-Phenylalamine	2 g
Disodium Hydrogen Phosphate	L g
Sodium Chloride	5 g
Agar	12g
Distilled Water	1 Lr
Ph	7.4

Distributed in tubes and sterilized by autoclaving at 121° c for 1 5 minutes, allowed to solidify as long slopes.

11. Sugar Fermentation Medium

Peptone 15g	
Andrade's Indicator	10 ml
Sugar to be tested	20g
Water	1 litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to Which sufficient 1m sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and andrade's indicator in 1 litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted durham’s tube. Sterilize by steaming at 100 ° C for 30 minutes on 3 consecutive days.

ANNEXURE I: CERTIFICATE OF APPROVAL

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

IEC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. Swati Sahai,
Postgraduate M.D.(Microbiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr.Swati Sahai,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study on microbiological profile of symptomatic catheter associated urinary tract infection in intensive care unit setup in a tertiary care hospital". no.24102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.R.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
6. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
7. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
8. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
9. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS CO
MADRAS MEDICAL CO.
CHENNAI-6

ANNEXURE II

PROFORMA

- Name : IP no:
- Age: Ward:
- Sex: Date of admission:
- Occupation:
- Address:

Indication for Foley's Catheter:

Date of Foley's catheter insertion:

Date of Foley's catheter removal/change:

Total catheter days:

Aseptic technique of insertion (Y/N):

Disconnection of drainage system:

Faulty catheter care(Y/N):

Development of following symptoms:

Symptom	Day of development/ presence	Sample collection day

Fever		
Chills		
Suprapubic pain		
Back pain (costovertebral angle pain):		
Dysuria		
Frequency		
Urgency		

Present history (medical & surgical):

Past history (medical & surgical):

Associated impaired state:

- Tuberculosis
- Chronic steroid intake
- Diabetes mellitus
- HIV
- Other:

Other evidence of infection found on direct exam or by diagnostic tests:

Provisional diagnosis:

Laboratory evaluation:

Biochemical parameters:

- Plasma glucose levels
- Blood urea
- Creatinine

Hematological investigations:

- TC
- DC
- Hb estimation
- ESR
- Other tests:

Microbiological investigation:

Sample (catheterized urine) collection date/day	Apperance of urine	Direct Examination			Culture	
		Gram stain	Wet mount	Dipstick test for urine nitrite/ leukocyte esterase	MAC	BAP

Colony Morphology In MAC : BAP : **Gram Staining Reaction:**	
Bacterial isolate: Motility of the Organism: Catalase Oxidase Coagulase: Slide coagulase Tube coagulase Bile esculin Biochemical Reactions: Indole Test Triple sugar iron media	**Fungal (candida) isolate:** Germ tube test: Chrom agar: Sugar assimilation test: Sugar fermentation test:

Simmons' citrate medium Christensen's urease medium Other tests:	Other tests:
Organism(s) isolated:	
Antibiogram:	

ANNEXURE III

CONSENT FORM

STUDY TITLE:

"A study on microbiological profile of symptomatic catheter associated urinary tract infection in intensive care unit setup in a tertiary care hospital''

Name: Date:

Age : IP No :

Sex : Project Patient No. :

Documentation of the informed consent

I, _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I hereby give my consent to be included as a participant in **"A study on microbiological profile of symptomatic catheter associated urinary tract infection in intensive care unit setup in a tertiary care hospital''**

I have read and understood this consent form and the information provided to me.

1. I have had the consent document explained to me.
2. I have been explained about the nature of the study.
3. I have been explained about my rights and responsibilities by the investigator.

4. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
5. I have been advised about the risks associated with my participation in this study.
6. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
7. I have not participated in any research study within the past _____ month(s).
8. I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.
9. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.
10. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
11. I have understand that my identity will be kept confidential if my data are publicly presented.
12. I have had my questions answered to my satisfaction.
13. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For participants:

Name and signature / thumb impression of the participant

OR legal representative if participant incompetent/For age 10-17 yrs-Name& signature of the parent/guardian.

Name _____

Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____

Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____

Signature_____

Date_____

ANNEXURE IV

MASTER CHART

S.no.	IP no.	Age	Gender	Total catheter days	Indication for catheterisation	Indication valid (Y/N)	Symptomatic CAUTI		Day of development of Symptomatic CAUTI						Risk factors present										Organism isolated	Antimicrobial susceptibility																				ESBL	Amp C	MBL	Carbapenemase	MRSA
			M/F				Y	N	3	5	7	10	14	21	Age ≥ 50 yrs	Female sex	Duration of catheterisation ≥ 10 days	Diabetes mellitus	Neurological causes	Respiratory causes	Urological/Nephrological causes	Steroid	Other immunocompromised conditions	FauLty catheter care		Amp	Ak	Gm	Nt	Nx	Cotri	Ctx	Caz	PT	Ipm	Mrp	Tetra	Pen	Vanco	HLG	Cx	Flu	Vori	Ampho	Itra					
1	131216	58	M	8	monitor urinary output	Y		N							Y	N	N	N	N	N	N	N	N	N																									
2	132056	52	F	14	monitor urinary output	Y	Y						Y		Y	Y	Y	N	N	Y	N	N	N	N	E.coli	R	R	S	S	R	R	R	R	S	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
																									Enterococcus faecalis	–	*	*	R	R	*	*	*	–	–	–	R	R	S	S	*	–	–	–	–	–	–	–	–	–
3	131668	70	M	10	monitor urinary output	Y		N							Y	N	Y	N	N	Y	N	N	N	N																									
4	132881	20	F	9	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
5	132282	32	F	9	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
6	132341	65	F	8	monitor urinary output	Y		N							Y	Y	N	Y	N	N	N	N	N	N																									
7	131564	68	M	8	monitor urinary output	Y		N							Y	N	N	N	Y	N	N	N	N	N																									
8	132762	45	M	10	monitor urinary output	Y	Y					Y			N	N	Y	N	Y	N	N	N	N	N	K.pneumoniae	*	R	R	R	R	R	R	R	R	R	R	R	–	–	–	R	–	–	–	–	N	N	Y	Y	–
9	132844	21	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
10	58	33	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
11	94766	20	F	21	monitor urinary output	Y	Y							Y	N	Y	Y	N	Y	N	N	N	N	N	K.pneumoniae	*	R	R	R	R	R	R	R	R	R	R	R	–	–	–	R	–	–	–	–	N	N	Y	Y	–
																									Enterococcus faecalis	–	*	*	S	S	*	*	*	–	–	–	R	R	S	R	*	–	–	–	–	–	–	–	–	–
12	253	35	M	10	monitor urinary output	Y		N							N	N	Y	N	N	N	N	N	N	N																									
13	9480	80	F	10	monitor urinary output	Y	Y						Y		Y	Y	Y	Y	N	N	N	N	N	N	P.aeruginosa	*	R	R	R	R	*	*	R	S	S	S	*	–	–	–	S	–	–	–	–	Y	N	N	N	–
																									Candida krusei	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	S	S	S	–	–	–	–	–
14	6296	53	M	7	monitor urinary output, relieve urinary retention	Y		N							Y	N	N	N	N	N	Y	N	N	N																									
15	10342	30	F	9	monitor urinary output	Y		N							N	Y	N	Y	N	N	N	N	N	N																									
16	10019	38	M	7	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
17	10557	39	F	8	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
18	132256	45	M	14	monitor urinary output	Y	Y						Y		N	N	Y	N	Y	N	N	N	N	N	Staphylococcus aureus	–	R	R	S	R	R	–	–	–	–	–	S	R	S	–	R	–	–	–	–	–	–	–	–	Y
19	10302	27	M	7	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
20	7766	72	M	7	monitor urinary output	Y		N							Y	N	N	N	N	N	N	N	N	N																									
21	8638	45	F	14	monitor urinary output	Y	Y						Y		N	Y	Y	N	Y	N	N	N	N	N	P.stutzeri	*	R	R	R	R	R	R	R	R	S	S	R	–	–	–	R	–	–	–	–	N	Y	N	N	–
22	19425	38	M	9	monitor urinary output	Y		N							N	N	N	Y	N	N	N	N	N	N																									
23	10144	33	M	7	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
24	667	67	F	14	monitor urinary output	Y	Y						Y		Y	Y	Y	Y	Y	N	N	N	N	N	Enterococcus faecalis	–	*	*	S	R	*	*	*	–	–	–	R	R	S	S	–	–	–	–	–	–	–	–	–
25	9407	33	F	10	monitor urinary output	Y		N							N	Y	Y	N	Y	N	N	N	N	N																									
26	8640	24	M	14	monitor urinary output	Y	Y						Y		N	N	Y	N	Y	N	N	N	N	N	Candida albicans	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S	S	S	S	–	–	–	–	–
																									Enterococcus faecalis	–	*	*	S	S	*	*	*	–	–	–	S	R	S	S	S	–	–	–	–	–	–	–	–	–
27	8986	52	F	14	monitor urinary output	Y	Y						Y		Y	Y	Y	N	N	N	N	N	N	N	P.fluorescens	*	R	R	R	R	R	R	R	S	S	S	R	–	–	–	S	S	–	–	–	Y	N	N	N	–
																									K.pneumoniae	*	R	R	S	R	R	R	R	R	S	S	R	–	–	–	R	–	–	–	–	N	Y	N	N	–
28	10589	48	M	9	monitor urinary output	Y		N							N	N	N	N	N	N	N	Y	N	N																									
29	11026	19	F	13	monitor urinary output	Y		N							N	Y	Y	N	N	N	N	Y	Y	N																									
30	6749	74	M	10	monitor urinary output	Y	Y					Y			Y	N	N	N	N	N	N	N	N	N	K.oxytoca	R	R	R	S	R	R	R	R	S	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
31	9926	32	F	16	monitor urinary output	Y		N							N	Y	Y	N	N	N	N	N	N	N																									
32	11807	62	M	6	monitor urinary output	Y		N							Y	N	N	Y	N	N	N	N	Y	N																									
33	11459	23	F	14	monitor urinary output	Y	Y						Y		N	Y	Y	Y	N	N	N	N	N	N	E.coli	R	R	R	R	R	R	R	R	S	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
																									Candida parapsilosis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S	S	S	S	–	–	–	–	–
34	12086	25	F	7	monitor urinary output	Y		N							N	Y	N	N	N	N	N	Y	Y	N																									
35	12221	34	F	9	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
36	13131	29	F	8	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
37	13129	20	M	14	monitor urinary output	Y	Y						Y		N	N	Y	N	N	Y	N	N	N	N	P.aeruginosa	*	R	R	R	S	*	*	R	S	S	S	*	–	–	–	S	–	–	–	–	Y	N	N	N	–
38	13256	45	M	9	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
39	11787	41	M	14	monitor urinary output	Y	Y						Y		N	N	Y	N	N	Y	N	N	N	N	P.aeruginosa	*	R	S	R	S	*	*	S	S	S	S	*	–	–	–	S	–	–	–	–	Y	N	N	N	–
																									Candida tropicalis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S	S	S	S	–	–	–	–	–
40	10738	65	F	8	monitor urinary output	Y		N							Y	Y	N	N	N	N	N	N	N	N																									
41	13165	61	M	9	monitor urinary output	Y		N							Y	N	N	N	N	N	N	N	N	N																									
42	12231	28	F	8	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
43	16431	27	F	14	monitor urinary output	Y	Y						Y		N	Y	Y	N	N	N	N	N	N	N	Enterococcus faecalis	–	*	*	S	R	*	*	*	–	–	–	R	R	S	S	–	–	–	–	–	–	–	–	–
44	11166	26	M	7	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
45	71931	52	M	8	monitor urinary output	Y		N							Y	N	N	Y	Y	N	N	N	N	N																									
46	12684	29	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
47	11041	35	F	7	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
48	12143	35	F	14	monitor urinary output	Y	Y						Y		N	Y	Y	N	N	N	N	Y	Y	N	K.pneumoniae	*	R	R	R	R	R	R	R	R	S	S	R	–	–	–	S	–	–	–	–	N	Y	N	N	–
																									P.stutzeri	*	R	R	R	R	R	R	R	S	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
49	11822	68	F	8	monitor urinary output	Y		N							Y	Y	N	Y	N	Y	N	N	N	N																									
50	7139	34	F	14	monitor urinary output	Y	Y						Y		N	Y	Y	Y	N	N	N	Y	N	N	E.coli	R	R	R	R	R	R	R	R	R	S	S	R	–	–	–	R	–	–	–	–	N	Y	N	N	–
																									K.pneumoniae	*	R	R	R	R	R	R	R	R	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
51	11057	54	M	10	monitor urinary output	Y		N							Y	N	Y	N	Y	N	N	N	N	N																									
52	13824	55	M	8	monitor urinary output	Y		N							Y	N	N	N	N	N	N	N	N	N																									
53	14510	45	F	10	monitor urinary output	Y		N							N	Y	Y	N	Y	N	N	N	N	N																									
54	14644	32	M	14	monitor urinary output	Y	Y						Y		N	N	Y	Y	N	Y	N	N	N	N	P.aeruginosa	*	R	R	R	R	*	*	R	R	S	S	*	–	–	–	R	–	–	–	–	N	Y	N	N	–
																									C.glabrata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S	S	S	S	–	–	–	–	–
55	65230	38	M	7	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
56	166211	22	F	9	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
57	11214	35	M	10	monitor urinary output	Y		N							N	N	Y	N	Y	N	N	N	N	N																									
58	4899	45	F	21	monitor urinary output, relieve urinary retention	Y	Y							Y	N	Y	Y	Y	N	N	Y	N	N	N	P.aeruginosa	*	R	R	R	R	*	*	R	R	R	R	*	–	–	–	R	–	–	–	–	N	N	Y	Y	–
																									K.pneumoniae	*	R	R	R	R	R	R	R	S	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
59	13894	55	M	10	monitor urinary output	Y		N							Y	N	Y	N	Y	N	N	N	N	N																									
60	17400	30	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
61	17351	27	M	7	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
62	15889	42	F	14	monitor urinary output	Y	Y						Y		N	Y	Y	N	N	Y	Y	N	N	N	Enterococcus faecalis	–	*	*	R	S	*	*	*	–	–	–	R	R	S	S	–	–	–	–	–	–	–	–	–
63	16330	50	M	8	monitor urinary output	Y		N							Y	N	N	N	Y	N	N	N	N	N																									
64	17385	21	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
65	16839	30	F	8	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
66	13129	51	F	10	monitor urinary output	Y	Y				Y				Y	Y	Y	N	N	Y	N	N	N	N	P.aeruginosa	*	R	R	R	R	*	*	R	R	S	S	*	–	–	–	R	–	–	–	–	N	Y	N	N	–
67	17343	33	F	9	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
68	17369	18	M	10	monitor urinary output	Y		N							N	N	Y	N	N	N	N	N	N	N																									
69	16805	46	M	9	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
70	15959	40	M	14	monitor urinary output	Y	Y						Y		N	N	Y	Y	Y	N	N	N	Y	N	P.aeruginosa	*	R	R	R	R	*	*	R	S	S	S	*	–	–	–	S	–	–	–	–	Y	N	N	N	–
71	16400	65	M	10	monitor urinary output	Y		N							Y	N	N	N	N	N	N	N	N	N																									
72	16681	46	M	10	monitor urinary output	Y		N							N	N	Y	N	N	N	N	N	N	Y																									
73	20451	25	M	9	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
74	19249	20	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
75	8981	19	F	8	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
76	20269	31	M	14	monitor urinary output, relieve urinary retention	Y	Y						Y		N	N	Y	N	N	N	Y	N	N	N	P.aeruginosa	*	R	R	R	R	*	*	R	S	S	S	*	–	–	–	S	–	–	–	–	Y	N	N	N	–
																									C.tropicalis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S	S	S	S	–	–	–	–	–
77	20070	55	F	9	monitor urinary output	Y		N							Y	Y	N	Y	N	N	N	N	N	N																									
78	20001	18	F	9	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
79	20113	25	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
80	19301	42	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
81	19335	40	M	10	monitor urinary output	Y		N							N	N	Y	N	N	N	N	N	N	N																									
82	14588	68	F	21	monitor urinary output, relieve urinary retention	Y	Y							Y	Y	Y	Y	N	Y	N	Y	N	N	N	E.coli	R	R	R	S	R	R	R	R	S	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
83	20094	19	F	9	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
84	19525	58	M	9	monitor urinary output	Y		N							Y	N	N	N	N	N	N	N	N	N																									
85	19506	32	F	8	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
86	16706	22	M	9	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
87	20968	45	F	14	monitor urinary output	Y	Y						Y		N	Y	Y	Y	Y	Y	N	N	N	N	P.aeruginosa	*	R	R	R	R	*	*	R	S	S	S	*	–	–	–	S	–	–	–	–	Y	N	N	N	–
																									E.coli	R	R	R	S	R	R	R	R	S	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
88	51527	35	F	10	monitor urinary output	Y		N							N	Y	Y	N	N	N	N	N	N	N																									
89	52060	20	M	12	monitor urinary output	Y		N							N	N	Y	N	N	Y	N	N	N	N																									
90	20480	47	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
91	50432	42	M	10	monitor urinary output	Y	Y				Y				N	N	Y	Y	N	N	N	N	N	Y	K.pneumoniae	*	R	R	R	S	R	R	R	S	S	S	S	–	–	–	S	–	–	–	–	Y	N	N	N	–
92	45141	27	F	8	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
93	48796	42	M	11	monitor urinary output	Y		N							N	N	Y	N	N	N	N	N	N	N																									
94	50439	46	M	12	monitor urinary output	Y		N							N	N	Y	N	N	N	N	N	N	N																									
95	47335	74	M	14	monitor urinary output	Y	Y						Y		Y	N	Y	Y	Y	N	N	N	N	N	P.aeruginosa	*	S	R	R	R	*	*	R	S	S	S	*	–	–	–	S	–	–	–	–	Y	N	N	N	–
																									E.coli	R	R	R	R	R	R	R	R	S	S	S	R	–	–	–	S	–	–	–	–	Y	N	N	N	–
96	49509	23	F	9	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									
97	65352	20	M	9	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	Y																									
98	64550	24	M	8	monitor urinary output	Y		N							N	N	N	N	N	N	N	N	N	N																									
99	64818	40	M	9	monitor urinary output	Y		N							N	N	N	N	N	Y	N	N	N	N																									
100	64267	35	F	8	monitor urinary output	Y		N							N	Y	N	N	N	N	N	N	N	N																									

KEY TO MASTER CHART

Ak	Amikacin
Amp	Ampicillin
Ampho	Amphotericin B
Cotri	Cotrimoxazole
Ctx	Cefotaxime
Caz	Ceftazidime
Cx	Cefoxitin
ESBL	Extended spectrum betalactamase
F	Female
Flu	Fluconazole
Gm	Gentamicin
HLG	High level gentamicin
Ipm	Imipenem
Itra	Itraconazole
M	Male
MBL	Metallobetalactamase
MRSA	Methicillin resistant *Staphylococcus aureus*
Mrp	Meropenem
Nor	Norfloxacin
Nit	Nitrofurantoin
Pen	Penicillin
PT	Piperacillin-tazobactam

Y	Yes
N	No
R	Resistant
S	Sensitive
Tetra	Tetracycline
Van	Vancomycin
Vori	Voriconazole
*	intrinsic resistance
-	not applicable

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